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ANNALS

OF THE

MISSOURI BOTANICAL GARDEN

ERRATA

Page 278, 6 lines from bottom—for **viscossima**, read **viscosissima.**

Page 278, 7 lines from bottom—for *viscossima*, read *viscosissima.*

Page 297, next to last line of footnote—for Cotton, read Colton.

Page 320, line 2—for ½, read 1–2.

Page 328, line 13—for p. 335, read p. 333.

Page 330, first line, last paragraph—for pl. 13, read fig. 34.

Page 354, line 5—for page 335, read page 333.

Page 365, line 2—for southeastern, read southwestern.

Annals
of the
Missouri Botanical Garden

Volume XXXII
1945

With 19 Plates and 147 Figures

Published quarterly at Galesburg, Illinois, by the Board of Trustees of the Missouri Botanical Garden, St. Louis, Mo.

Entered as second-class matter at the post-office at Galesburg, Illinois, under the Act of March 3, 1879.

64339

Annals
of the
Missouri Botanical Garden

A Quarterly Journal containing Scientific Contributions from the Missouri Botanical Garden and the Henry Shaw School of Botany of Washington University in affiliation with the Missouri Botanical Garden.

Information

The ANNALS OF THE MISSOURI BOTANICAL GARDEN appears four times during the calendar year: February, April, September, and November. Four numbers constitute a volume.

Subscription Price	$10.00 per volume
Single Numbers	2.50 each

Contents of previous issues of the ANNALS OF THE MISSOURI BOTANICAL GARDEN are listed in the Agricultural Index, published by the H. W. Wilson Company.

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FLORA OF PANAMA

BY

ROBERT E. WOODSON, Jr.

AND

ROBERT W. SCHERY

AND COLLABORATORS

PART III

Fascicle 1

JUNCACEAE
LILIACEAE
SMILACACEAE (Morton)
HAEMODORACEAE
AMARYLLIDACEAE
VELLOZIACEAE
DIOSCOREACEAE (Morton)
IRIDACEAE
BURMANNIACEAE (Jonker)
MUSACEAE
ZINGIBERACEAE
CANNACEAE
MARANTACEAE

ANNALS

OF THE

MISSOURI BOTANICAL GARDEN

Vol. XXXII FEBRUARY, 1945 No. 1

FLORA OF PANAMA

Part III. Fascicle 1

JUNCACEAE

Perennial, rarely annual, grass-like plants with stoloniferous rhizomes; stems scapiform, foliate chiefly at the base, the upper leaves usually reduced and distant; leaves narrow, sheathed at the base, the sheath open or closed; inflorescence terminal, paniculate (in Panama), bracteate; flowers small, actinomorphic, perfect; perianth of 6 equal glumaceous tepals; stamens 6, hypogynous, free; anthers basifixed, 2-celled; pistil superior, 1- to 3-celled; stigmas 3; capsule loculicidal, seeds 3 to numerous.

1. LUZULA DC.

LUZULA DC. in Lam. & DC. Fl. Fr. 1:198. 1805; Buchenau in Engl. Pflanzenreich IV. 36:42. 1906.

Stoloniferous, tufted perennials; stems scapiform, foliate chiefly in a basal rosette; leaves grass-like, the basal sheaths closed; inflorescence paniculate, bearing very many small, glumaceous, perfect flowers.

1. LUZULA GIGANTEA Desv. in Jour. de Bot. 1:145. 1808; Buchenau in Engl. Pflanzenreich IV. 36:59. 1906.

Luzula paniculata Desv. loc. cit. 147. *pl. 5.* 1808.

Luzula latifolia Liebm. in Vid. Meddel. Nat. Foren. Kjöbenh. 47. 1850.

Juncodes giganteum (Desv.) Sheldon in Minnesota Bot. Stud. 1:62. 1894.

Luzula gigantea var. *vulcanica* Woodson in Ann. Missouri Bot. Gard. 26:275. 1939.

Fig. 1. *Luzula gigantea*

Stems erect or ascending, 1.5–9.0 dm. tall in flower; basal leaves rather broadly lance-ensiform, 5–30 cm. long, conspicuously subarachnoid-ciliate to glabrate; inflorescence broadly and rather diffusely paniculate, the minute florets scarious, shining, deep chestnut-brown.

Mexico to Bolivia, in mountain meadows and open forest.

Issued February 28, 1945.

CHIRIQUÍ: Potrero Muleto to summit, Volcán de Chiriquí, *Maxon 5361, Woodson & Schery 432, Woodson, Allen & Seibert 1094.*

The genus *Juncus,* with narrower leaves and open sheaths, almost certainly occurs in the mountains of Panama, although it has not as yet been collected. Three species have been reported from Costa Rica.

LILIACEAE

Chiefly rhizomatous or bulbous perennial herbs; leaves usually alternate or radical, occasionally approximate or whorled; inflorescence various, usually racemose or umbelliform in Panama; flowers 6-merous (rarely 2- to 4- to 5-merous); perianth of 6 free or united, chiefly petalaceous segments; stamens as many as the perianth segments, the filaments free, the anthers free or coherent, chiefly versatile, dehiscing longitudinally; pistil 3-celled, each cell containing 1 to several ovules; fruit usually capsular, occasionally baccate.

a. Leaves chiefly in a basal rosette, those of the flowering scapes greatly reduced; rhizome short, vertical; fruit a capsule.
  b. Anthers free ..... 1. ANTHERICUM
  bb. Anthers coherent ..... 2. ECHEANDIA
aa. Leaves all cauline; rhizome more or less elongate, horizontal; fruit a berry ..... 3. SMILACINA

In Panama one may find numerous exotic genera of Liliaceae in cultivation, occasionally as escapes. These include *Allium, Asparagus, Agapanthus, Aloe, Dracaena, Sansevieria,* and *Taetsia.*

1. ANTHERICUM L.

ANTHERICUM L. Sp. Pl. 310. 1753; Krause in Engl. & Prantl, Nat. Pflanzenfam. 15a:282. 1930.

Stellarioides Medic. Acta Acad. Theod.-palat. 6:Phys. 369. 1790.
Phalanganthus Schrank, apud Haw. Syn. Pl. Succ. 67. 1819.
Blephanthera Raf. Fl. Tellur. 2:59. 1836.
Endogona Raf. loc. cit. 27. 1836.
Obsitila Raf. loc. cit. 1836.
Trachinema Raf. loc. cit. 1836.
Licinia Raf. loc. cit. 3:57. 1836.

Subscapose herbs from a relatively short vertical rhizome producing numerous fleshy roots; leaves chiefly in a basal rosette, those of the flowering stem remote and greatly reduced, narrowly lanceolate to linear, parallel-veined; inflorescence terminating the flowering scape, simply racemose or occasionally branched at the base; flowers solitary or in pairs or small fascicles in the axils of scarious or slightly foliaceous bracts; perianth segments 6, free and essentially equal, 3- to 7-nerved; stamens 6, hypogynous, the anthers free at anthesis, sagittate; pistil 3-celled, the style simple, the stigma subcapitate; fruit a 3- to several-seeded capsule.

a. Flowers yellow, 1.2–1.5 cm. long ..... 1. A. ECHEANDIOIDES
aa. Flowers white, 0.6–0.7 cm. long ..... 2. A. MACROPHYLLUM

1. ANTHERICUM ECHEANDIOIDES Baker in Bot. Mag. *pl. 6809.* 1885.

Anthericum apodastanthum Donn. Sm. in Bot. Gaz. 19:265. 1894.

Basal leaves linear, 17–25 cm. long, 0.8–1.0 cm. broad, glabrous; flowering scapes 24–36 cm. long, bearing several rather distant, reduced leaves or bracts; flowers showy, solitary or in small fascicles in the axils of small, scarious bracts; pedicels about 1 cm. long, articulated toward the base; perianth segments oblong-elliptic, 1.2–1.5 cm. long, bright yellow with 3 brown nerves; anthers 0.5 cm. long.

Southern Mexico to Costa Rica and Panama, in subalpine meadows.

CHIRIQUÍ: upper Río Chiriquí Viejo valley, *P. White 32.*

2. ANTHERICUM MACROPHYLLUM Baker in Engl. Bot. Jahrb. 8:209. 1887.

Anthericum panamense Standl. in Field Mus. Publ. Bot. 22:327. 1940.

Fig. 2. *Anthericum macrophyllum*

Basal leaves ensiform, long-acuminate, 15–30 cm. long, 1–2 cm. broad, multinerved, glabrous; flowering scapes 25–35 cm. long, simple or infrequently branched at the base, bearing many small white flowers; flowering bracts 0.3–1.0 cm. long, slightly foliaceous; pedicels 0.3–0.5 cm. long, articulated at the middle; perianth segments 0.6–0.7 cm. long; capsules broadly trigonal, 0.5–0.6 cm. long.

Costa Rica and Panama, in highland forests.

COCLÉ: El Valle de Antón, *Allen 1905, Woodson & Schery 178.*

2. ECHEANDIA Ortega

ECHEANDIA Ortega, Nov. Pl. Descr. 90, 135. *t. 18.* 1797; Weatherby in Proc. Amer. Acad. 45:388. 1910.

Echeandiaea Post & O. Ktze. Lexic. Gen. Phan. 23. 1903.

Subscapose herbs from a rather short vertical rhizome, producing numerous fleshy, frequently tuberiferous roots; leaves chiefly in a basal rosette, those of the flowering scape remote and greatly reduced, narrowly lanceolate or ensiform, parallel-veined; inflorescence terminating the flowering scape, paniculately branched or infrequently simple and racemiform; flowers solitary or fasciculate; perianth segments 6, free, essentially equal; stamens 6, the anthers strongly connate at anthesis, sagittate; pistil 3-celled, the style simple, the stigma subcapitate; fruit a 3- to several-seeded loculicidal capsule.

Fig. 3. *Echeandia venusta*

a. Flowers rather showy, the perianth segments oblong-elliptic, 1.5–2.0 cm. long, bright yellow ..... 1. E. VENUSTA
aa. Flowers inconspicuous, the perianth segments linear-lanceolate, 1.0–1.3 cm. long, white ..... 2. E. PROLIXA

1. ECHEANDIA VENUSTA Woodson in Ann. Missouri Bot. Gard. 29:325. 1942.

Perennial herbs 3–4 dm. tall, with short vertical rhizomes and clusters of fleshy subtuberous roots, glabrous throughout; leaves mostly radical, broadly linear, 12–30 cm. long, 1.0–1.5 cm. broad, white-margined; inflorescence racemiform, usually simple, the flowers in fascicles or solitary, subtended by very conspicuous foliaceous or spathaceous bracts 2–7 cm. long; pedicels 1.5–2.0 cm. long, articulated below the middle; perianth segments oblong-elliptic, 1.5–2.0 cm. long, golden yellow with 3 black nerves; staminal filaments about 0.5 cm. long, rugose, the anthers narrowly sagittate, about 0.6 cm. long.

Panama, in alpine meadows.

CHIRIQUÍ: Potrero Muleto, Volcán de Chiriquí, *Woodson & Schery 379.*

2. ECHEANDIA PROLIXA Woodson in Ann. Missouri Bot. Gard. 29:35. 1942.

Perennial herbs 8–12 dm. tall, with short vertical rhizomes and clusters of fleshy, tuberiferous roots, glabrous throughout; leaves mostly radical, broadly linear, 60–95 cm. long, about 2 cm. broad; inflorescence diffuse and usually more or less procumbent, paniculate-racemiform; flowers small, gathered into small fascicles subtended by small scarious bracts; pedicels 1.0–1.5 cm. long, articulated below the middle; perianth segments linear-lanceolate, 1.0–1.3 cm. long, white; anthers oblong-sagittate, 0.6 cm. long, the filaments of about equal length, rugose; capsules obovoid-oblongoid, truncate or slightly emarginate at the tip, narrowed toward the base, 0.7–0.8 cm. long, about 0.4 cm. broad.

Panama, on rocky hilltops near sea-level.

PANAMÁ: vicinity of Bejuco, *Allen 2962.*

3. SMILACINA Desf.

SMILACINA Desf. in Ann. Mus. Paris 9:51. *t. 9.* 1807, *nom. conserv.*
Vagnera Adans. Fam. Pl. 2:496. 1763, *nom. rejic.*
Tovaria Neck. Elem. 2:190. 1790; Krause in Engl. & Prantl, Nat. Pflanzenfam. 15a:367. 1930, *nom. rejic.*
Polygonastrum Moench, Meth. 637. 1794, *nom. rejic.*
Sigillaria Raf. in Jour. Phys. 89:261. 1819.
Styrandra Raf. loc. cit. 102. 1819.
Asteranthemum Kunth, Enum. Pl. 5:151. 1850.
Jocaste Kunth, loc. cit. 154. 1850.
Medora Kunth, loc. cit. 155. 1850.
Neolexis Salisb. Gen. Pl. 64. 1866.

Mediocre to massive, caulescent herbs from a relatively elongate fleshy horizontal rhizome; leaves all cauline, alternate or approximate, parallel-veined; inflorescence a many-flowered, terminal panicle; perianth segments 6, equal, free; stamens 6, epipetalous; pistil superior, 3-celled; style terminal; stigma subcapitate; fruit a 1- to 6-seeded berry.

1. SMILACINA PANICULATA Mart. & Gal. in Bull. Acad. Brux. 9^2:388. 1842.

Tovaria thyrsoidea Baker in Jour. Linn. Soc. Bot. 14:568. 1875.
Tovaria laxiflora Baker, loc. cit. 569. 1875.
Tovaria nervulosa Baker, loc. cit. 1875.
Smilacina thyrsoidea (Baker) Hemsl. Biol. Centr.-Am. Bot. 3:368. 1884.
Smilacina laxiflora (Baker) Hemsl. loc. cit. 1884.
Smilacina nervulosa (Baker) Hemsl. loc. cit. 1884.

Fig. 4. *Smilacina paniculata*

Smilacina Gigas Woodson in Ann. Missouri Bot. Gard. 27:270. 1940.

Plants terrestrial, rarely epiphytic, 0.4–3.0 m. tall, glabrous; leaves all cauline, shortly petiolate, narrowly lanceolate to broadly ovate, acuminate, 6–30 cm. long; panicles 3–50 cm. long, 2–25 cm. broad, bearing many small white flowers; pedicels solitary, 1–10 mm. long, white or deep red; perianth segments ovate-lanceolate, 3–5 mm. long; berries globular, 0.3–0.4 cm. thick.

Mexico to Panama, in mountain forests.

CHIRIQUÍ: valley of upper Río Chiriquí Viejo, *Allen 1392;* from Cerro Punta to headwaters of Río Caldera, *Allen 1446;* Bajo Chorro, *Davidson 53;* Volcán de Chiriquí, *Killip 360, Pittier 3071, Woodson, Allen & Seibert 852;* Bajo Mona and Quebrada Chiquero, *Woodson & Schery 512;* Cerro Copete, *Woodson & Schery 339.*

Abundant in the highland forests of Chiriquí, and extremely variable in size and general aspect; occasionally epiphytic.

SMILACACEAE[1]

By C. V. Morton

1. SMILAX L.

Smilax L. Sp. Pl. 1028. 1753; Apt, in Fedde Repert. Sp. Nov. 18:385-422. 1922; Killip & Morton in Carn. Inst. Publ. 461:255-296. 1936.

Woody vines from large rhizomes; leaves alternate, palmately nerved, the petiole sheathing at base, bearing a pair of tendrils at the apex of the sheath; flowers unisexual, borne in solitary axillary umbels, or the umbels pseudoracemose on short axillary branchlets; perianth segments 6, distinct, narrow, equal or nearly so; stamens 6, the filaments slender, free; styles 3; ovules solitary in the cells, pendulous; fruit a fleshy berry, commonly 1-seeded.

The rhizomes of certain species produce the well-known Sarsaparilla of commerce.

[1] Published by permission of the Secretary of the Smithsonian Institution.

a. Plants entirely glabrous, sometimes spiny.
  b. Staminate flowers 2.8 mm. long or less; leaves with reticulate secondary veins, usually aculeate on the nerves beneath; branchlets angular; peduncles shorter than the petioles ..... 1. S. SPINOSA
  bb. Staminate flowers 4 mm. long or more; leaves unarmed.
    c. Peduncles of pistillate umbels shorter than the subtending petioles, terete; stems terete; anthers shorter than the filaments; staminodia 3 ..... 2. S. DOMINGENSIS
    cc. Peduncles of pistillate umbels longer than the petioles, flattened; staminodia 6 (not known in S. *chiriquensis*).
      d. Anthers longer than the filaments; stems (at least the lower) terete.
        e. Staminate flowers sessile; staminate umbels solitary, axillary, long-peduncled; leaves 5-nerved (the outer nerves marginal), the secondary veins parallel ..... 3. S. SPISSA
        ee. Staminate flowers obviously pedicellate; staminate umbels borne on short, bracteate, raceme-like branchlets; leaves 7-nerved (the outer nerves marginal), the secondary veins prominently reticulate ..... 4. S. PANAMENSIS
      dd. Anthers much shorter than the filaments; stems sharply quadrangular ..... 5. S. CHIRIQUENSIS
aa. Plants hairy or, in S. *subpubescens*, glabrate at maturity, but then at least a few hairs persistent on the petioles, peduncles or pedicels, always unarmed; anthers shorter than the filaments.
  b. Branchlets obtusely quadrangular, glabrate at maturity; staminodia 6; inflorescence and young growth rusty-tomentose; peduncles shorter than the subtending petioles; leaves glabrate at maturity ..... 6. S. SUBPUBESCENS
  bb. Branchlets terete (except the lowermost), hairy even at maturity; staminodia 3 ..... 7. S. MOLLIS

Fig. 5. *Smilax spinosa*

1. SMILAX SPINOSA Mill. Gard. Dict. ed. 8, no. 8. 1768.

Smilax Houstoniana Steud. Nom. ed. 2. 2:599. 1841 (*illegit.*)
Smilax mexicana Griseb. ex Kunth, Enum. 5:167. 1850.
Smilax Wagneriana A. DC. Monogr. Phan. 1:143. 1878.

Stems aculeate or rarely unarmed, the upper 4- to 6-angled, often flexuous; petioles rarely over 1 cm. long; lower leaf blades ovate to broadly elliptic, up to 14 cm. long and 8 cm. wide, subcordate at base, the upper much smaller, acute at base, all 5-nerved, the veins prominently reticulate and elevated on both surfaces, often aculeate beneath; peduncles of staminate umbels solitary, up to 1 cm. long, shorter than the subtending petiole, flattened; pedicels slender, 5–13 mm. long, longer than the peduncle; perianth seg-

ments ovate-oblong or oblong, 2.8 mm. long or less, 1–1.4 mm. wide; filaments shorter or longer than the anthers; peduncles of pistillate umbels up to 9 mm. long, shorter than the subtending petiole, flattened; staminodia 3 or 6, minute; berries black, globose, 4–12 mm. thick.

Mexico, south to Panama.

CANAL ZONE: Río Cocoli, *P. White 143;* Ancón Hill, *Seibert 122;* Fort Lorenzo, *Piper 5954.* CHIRIQUÍ: Boquete, *Davidson 651.* COCLÉ: Penonomé, *R. S. Williams 241.* PANAMÁ: Taboga Island, *Woodson, Allen & Seibert 1481;* Punta Paitilla, *Standley 26306;* swamp east of Río Tecúmen, *Standley 26670;* between Las Sabanas and Matías Hernández, *Standley 31872;* Río Tapia, *Maxon & Harvey 6622;* near Vigía and San Juan, *Dodge, Steyermark & Allen 16594.* VERAGUAS: Cañazas, *Allen 157.*

The type of S. *Wagneriana* was collected by M. Wagner in the province of Chiriquí. The var. *compta* Killip & Morton (Carn. Inst. Publ. 461:264. 1936) is distinguished by the scabrous stems covered with minute setiform spinules, which are found also on the leaf blades. It has been collected only near Alhajuela, Chagres Valley, Prov. Panamá, by Pittier (no. *3487*). An insufficiently known plant is S. *lappacea* var. *ornata* Killip & Morton (op. cit. 289), described from sterile material collected at Gamboa, Canal Zone *(Heriberto 71)*. It resembles S. *spinosa,* but the leaves are narrower. The stems are spiny, but not scabrous as in S. *spinosa* var. *compta*. The leaves are aculeate beneath, and also conspicuously setulose on the veins.

2. SMILAX DOMINGENSIS Willd. Sp. Pl. 4:783. 1806.

Smilax Schlechtendalii Kunth, Enum. 5:224. 1850.

Stems all terete, smooth, the lower sparingly aculeate, the upper unarmed; petioles up to 1.6 cm. long; leaf blades ovate-lanceolate or ovate, usually not over 9 cm. long and 5 cm. wide, dark green and shining above, unarmed, 5-nerved, the outer nerves marginal, the veins elevated on both surfaces, reticulate; peduncles of staminate umbels solitary or in short, bracteate, axillary branchlets, 1–5 mm. long, much shorter than the subtending petiole; pedicels 4–7 mm. long; perianth segments ligulate, 4.5–6.5 mm. long, 1.2–1.5 mm. wide; filaments 3–4 mm. long, the anthers shorter, 1.2–2 mm. long; peduncles of pistillate umbels subterete, up to 7 mm. long, much shorter than the subtending petiole; pedicels longer than peduncle; staminodia 3, about 1 mm. long; fruiting pedicels 4–10 mm. long; fruits dull-red or brown, globose, 5–10 mm. in diameter.

Southeastern United States, West Indies, and Mexico to Panama.

PANAMÁ: Juan Díaz, *Standley 30633.* CANAL ZONE: Aspinwall, *Hayes 638.*

3. SMILAX SPISSA Killip & Morton in Carn. Inst. Publ. 461:273. 1936.

Upper stems slender, terete, not flexuous, unarmed; petioles up to 2 cm. long; leaf blades oblong, up to 16 cm. long and 6 cm. wide, acute at base, 5-nerved, the outer nerves marginal, the veins impressed above, elevated beneath, the secondary subparallel, not conspicuously reticulate; peduncle of staminate umbel solitary,

up to 4.5 cm. long, longer than the subtending petiole; pedicels obsolete; flowers numerous, crowded, the outer perianth segments 4 mm. long and 1.5 mm. broad, the inner somewhat smaller; filaments 1–1.5 mm. long, the anthers longer, 1.5–2 mm. long; fruiting peduncle solitary, 1.5–2.3 cm. long, terete, longer than the subtending petiole; berry red, large, up to 15 mm. in diameter.

Costa Rica and Panama.

CANAL ZONE: Barro Colorado Island, *Standley 31295, 31314, 40796, 40820, Bailey & Bailey 364, Shattuck 767, Wetmore & Woodworth 49;* between Gorgona and Gatún, *Pittier 2260.*

4. SMILAX PANAMENSIS Morong in Bull. Torr. Bot. Club 21:441. 1894.

Smilax ramonensis Apt in Fedde Repert. Sp. Nov. 18:405. 1922.

Stems terete, smooth, the lower armed with broad-based spines 2 cm. long, the upper unarmed; petioles up to 3 cm. long; leaf blades ovate-oblong or the upper lanceolate-oblong, up to 19 cm. long and 9.5 cm. wide, acute or obtuse at base, unarmed, 7-nerved, the outer nerves marginal, the secondary veins prominently reticulate; staminate umbels borne on short, bracteate, axillary branchlets, these often paired or clustered, the peduncle proper up to 2 cm. long, flattened; pedicels 5–8 mm. long; perianth segments 4–6 mm. long, 1.5–1.75 mm. wide; anthers 2–2.75 mm. long, slightly or much longer than the filaments; peduncles of pistillate umbels solitary or on short, bracteate, axillary branchlets, the peduncle proper up to 1.5 cm. long, flattened; staminodia 6; fruiting peduncles up to 2.5 cm. long, the pedicels 7–15 mm. long, bulbous at base; berries probably red, 7.5–10 mm. in diameter.

Honduras, Costa Rica and Panama.

CANAL ZONE: Gatún, *Hayes 63* (TYPE); Barro Colorado Island, *Shattuck 699, Wetmore & Abbe 168.* CHIRIQUÍ: Paso Ancho to Monte Lirio, *Allen 1584.*

5. SMILAX CHIRIQUENSIS Morton, in Woodson & Schery, Ann. Missouri Bot. Gard. 29:326. 1942.

Vine 25 feet long, the stems conspicuously and sharply quadrangular, pale yellowish, glabrous, sparingly aculeate; petioles elongate, those of the larger leaves 6 cm. long, glabrous, articulate at or above the middle; leaf blades ovate, up to 19 cm. long and 12 cm. wide, short-apiculate at apex, the larger cordate at base, the smaller truncate, all entire, glabrous, 9-nerved, the outer nerves marginal, the secondary veins conspicuously reticulate, elevated on both surfaces; staminate umbels borne on short, axillary branchlets, the leaves subtending the umbels well developed or reduced to sheaths only; peduncle 1–3 cm. long, glabrous, strongly flattened, longer than the subtending petiole; pedicels 5–11 mm. long, glabrous; perianth segments linear, 8–9 mm. long, about 1.5 mm. wide, glabrous; filaments about 6 mm. long, the anthers much shorter, about 1.5 mm. long; pistillate flowers and fruits unknown.

Confined to Panama.

CHIRIQUÍ: valley of upper Río Chiriquí Viejo, *P. White 348* (TYPE), *G. White 59*; Bajo Mona, Boquete, *Davidson 478*.

6. SMILAX SUBPUBESCENS A. DC. Monogr. Phan. 1:69. 1878.

Smilax calocardia Standl. in Field Mus. Publ. Bot. 22:7. 1940.

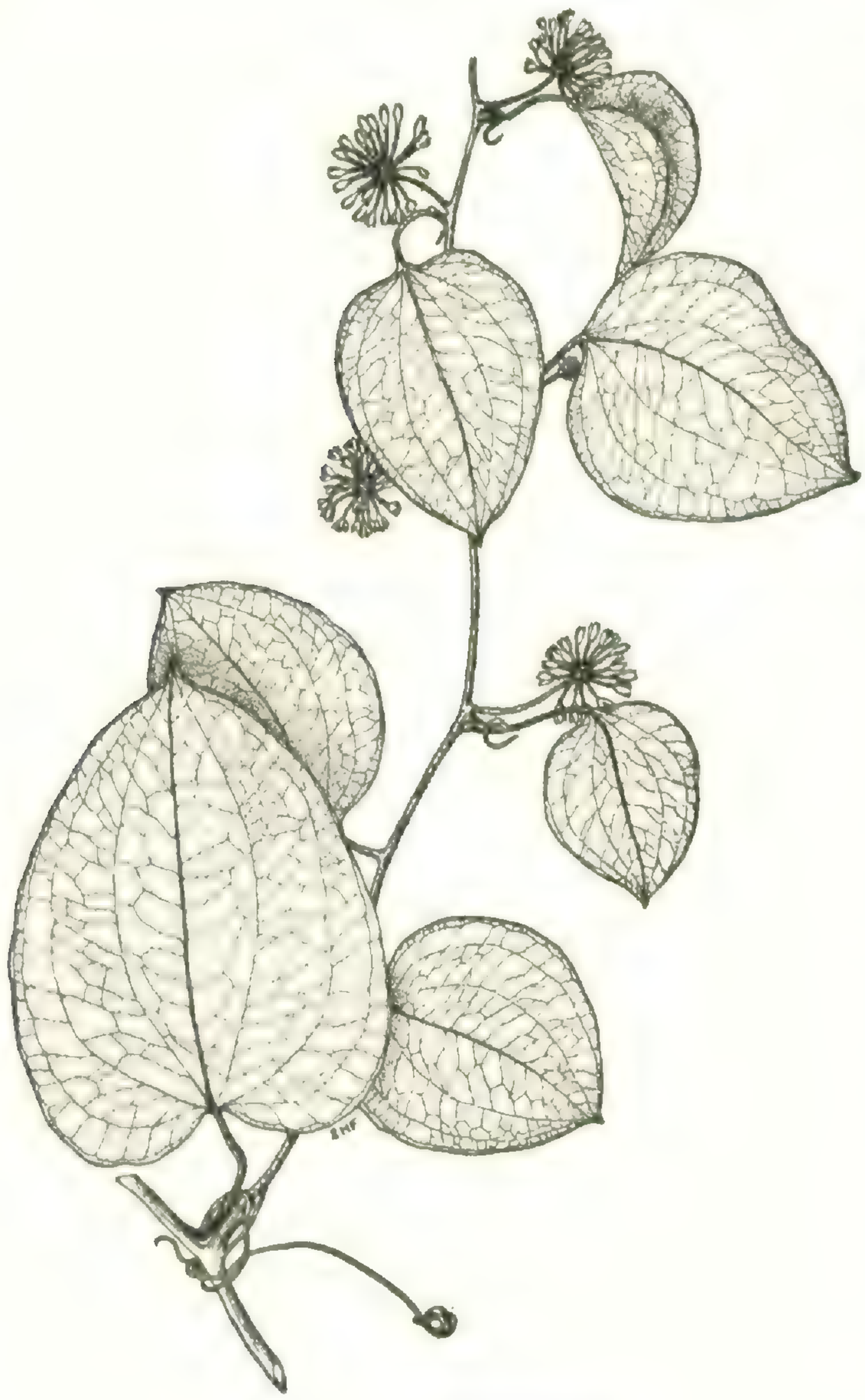

Fig. 6. *Smilax subpubescens*

Stems obtusely quadrangular, unarmed, tomentose when young, glabrate at maturity; petioles up to 7 cm. long, often persistently hairy, at least on sheath; leaf blades broadly ovate, up to 24 cm. long and 19 cm. wide, deeply and openly cordate at base, the upper leaves smaller, often merely rounded or subtruncate at base, all unarmed, densely reddish-tomentose on both sides when very young, the older leaves nearly glabrous, 9- to 11-nerved, the secondary veins prominently reticulate; peduncles of staminate umbels solitary, up to 3.5 cm. long, usually shorter than the subtending petiole, flattened, tomentulose when young; perianth segments narrowly oblong, 5–6 mm. long, 1–1.5 mm. wide, densely or sparsely tomentulose; filaments 2.5–4 mm. long, the anthers much shorter, 1.6–2 mm. long; peduncles of pistillate umbels about 1 cm. long, shorter than the subtending petiole; pedicels about 6 mm. long; staminodia 6; fruiting peduncles up to 2.5 cm. long; berries orange.

Mexico to Panama.

CHIRIQUÍ: Finca Lérida, *Woodson & Schery 218*; Finca Lérida to Peña Blanca, *Woodson & Schery 305, 306*; Potrero Muleto to summit of Volcán de Chiriquí, *Woodson & Schery 456*; Casita Alta, *Woodson, Allen & Seibert 799, 822, 974*; valley of upper Río Chiriquí Viejo, vicinity of Monte Lirio, *Seibert 270*; Volcán de Chiriquí, *Davidson 953* (TYPE of *S. calocardia*).

7. SMILAX MOLLIS Humb. & Bonpl. ex Willd. Sp. Pl. 4:785. 1806.

Stems terete, unarmed, pilosulous or subtomentose; petioles up to 1.8 cm.

long, densely pubescent; leaf blades ovate-oblong or oval, up to 18 cm. long and 10.5 cm. wide, the upper much smaller, all unarmed, cordate at base, persistently hirsutulous on veins beneath, 7-nerved, the two outer marginal, the secondary veins prominently reticulate; peduncle of staminate umbel up to 4 cm. long, much longer than the subtending petiole, terete, densely short-hirsute; pedicels 3–5 mm. long, hirsutulous or rarely glabrous; perianth segments oblong-linear, 4–5 mm. long, 1 mm. wide, sparingly hairy or glabrous except for a tuft of hairs at apex; filaments 2–3.5 mm. long, the anthers much shorter, 1–1.2 mm. long; peduncle of pistillate umbels up to 3 cm. long, terete or slightly flattened, densely short-hirsute, usually longer than the subtending petiole; pedicels 3–5 mm. long, hirsutulous; staminodia 3; berries red or yellow, 4–8 mm. in diameter.

Mexico to Panama.

CANAL ZONE: Fort Randolph, *Standley 28685;* Fort Sherman, *Standley 31103;* between France Field and Catival, *Standley 30310;* Obispo, *Standley 31754;* Barro Colorado Island, *Standley 31431, 40829, 40956;* Frijoles, *Allen 923.* CHIRIQUÍ: Boquete, *Davidson 775.* COCLÉ: Bismarck, *R. S. Williams 610.*

HAEMODORACEAE

1. XIPHIDIUM Aubl.

XIPHIDIUM Aubl. Pl. Guian. 1:33. *t. 11.* 1775.

Perennial herbs with more or less elongate horizontal rhizomes; stems erect or ascending, usually unbranched; leaves alternate, equitant, ensiform, with closed basal sheaths, parallel-nerved; inflorescence terminal, a panicle of simple scorpioid cymes, bearing numerous rather small white flowers; flowers perfect, regular; perianth segments 6, petalaceous, persistent; stamens 3; filaments free; anthers 2-celled, longitudinally dehiscent; pistil subinferior, 3-celled; fruit a many-seeded berry.

Fig. 7. *Xiphidium caeruleum*

1. XIPHIDIUM CAERULEUM Aubl. Pl. Guian. 1:33. *t. 11.* 1775.

Ixia Xiphidium Loefl. Iter Hisp. 179. 1758.
Xiphidium album Willd. Sp. Pl. 1:249. 1797.
Xiphidium floribundum Sw. Fl. Ind. Occ. 1:80. 1797.
Xiphidium giganteum Lindl. in Bot. Reg. *pl. 66.* 1846.

Plants somewhat Iris-like, 3–8 dm. tall; leaves 20–50 cm. long, 1.5–4.5 cm. broad, minutely dentate-lacerate toward the tip; panicles 7–30 cm. long, 3–12 cm. broad, bearing numerous small white flowers; peduncle usually scurfy-puberu-

lent; pedicels 0.3–0.4 cm. long; perianth segments 0.4–0.6 cm. long; berries dull red, globose, about 0.5 cm. in diameter.

Mexico to Brazil and Bolivia; Antilles. Very common in lowland forests, occasionally at higher altitudes.

BOCAS DEL TORO: Almirante, *von Wedel 10;* Water Valley, *von Wedel 1370;* Shepherd Island, *von Wedel 2692;* Changuinola valley, *Dunlap 250.* CANAL ZONE: Ancón Hill, *Seibert 384;* Las Cruces, *Seibert 580;* Río Pequení, *Dodge, Steyermark & Allen 16597;* Gatún Lake, *Standley 31407;* Culebra, *Pittier 4776.* CHIRIQUÍ: Boquete, *Woodson & Schery 760.* PANAMÁ: Río La Maestra, *Allen 67;* Río Tapía, *Standley 26135;* Río Chilibre, *Piper 5682;* Juan Díaz, *Killip 3108.*

Locally known as *Palma, Palmita,* and *Palma del norte.*

AMARYLLIDACEAE

Chiefly scapose herbs from bulbs, corms, or tuberous rhizomes, occasionally herbaceous vines or stout xerophytes; inflorescence various; flowers usually showy, perfect, regular (in Panama); perianth of 6 separate or united petaloid segments, occasionally with an annular corona; stamens 6, filaments hypogynous or inserted on the tube of the perianth; anthers 2-celled, basifixed or versatile, dehiscing longitudinally; pistil inferior (in Panama), 3-celled (rarely 1-celled); ovules numerous; fruit a capsule or a berry.

a. Stout acaulescent or subacaulescent xerophytes with coriaceous rigid leaves; inflorescence a massive terminal panicle.
  b. Perianth rotate, the segments barely united at the base; stamens shorter than the perianth ..... 1. FURCRAEA
  bb. Perianth funnel-shaped, the segments united into a definite basal tube; stamens conspicuously exserted ..... 2. AGAVE
aa. Herbaceous vines with leafy stems; inflorescence chiefly umbelliform, rarely 1-flowered; perianth segments free to the base ..... 3. BOMAREA
aaa. Scapose herbs with basal, usually succulent leaves; inflorescence umbelliform or 1-flowered; perianth segments united at the base.
  b. Perianth without a tube (but the ovary long-beaked and resembling a narrow tube in *Curculigo*); plants scatteringly long-pilose, especially the inflorescence.
    c. Fruit a capsule, not beaked ..... 4. HYPOXIS
    cc. Fruit fleshy and indehiscent, ending in a long beak ..... 5. CURCULIGO
  bb. Perianth with a manifest, usually elongate tube; plants glabrous.
    c. Corona absent, the staminal filaments terete or narrow to their base.
      d. Flowers in umbels of several, large and showy; plants usually massive ..... 6. CRINUM
      dd. Flowers solitary, rarely paired, rather small; plants small ..... 7. ZEPHYRANTHES
    cc. Corona present, the staminal filaments greatly dilated and petaloid at their base.
      d. Perianth segments broadly ovate or ovate-lanceolate, the corona annular; leaves broadly ovate, with a long narrow petiole ..... 8. EUCHARIS
      dd. Perianth segments narrowly linear, the corona scyphiform; leaves lorate, sessile ..... 9. PANCRATIUM

Amongst cultivated Amaryllids, the Tuberose, *Polianthes,* popularly known as *Nardo,* is very frequent. Other exotic genera of gardens include *Agave, Hippeastrum,* and *Sprekelia.*

1. FURCRAEA Vent.

FURCRAEA Vent. in Bull. Soc. Philom. 1:65. 1793.

Funium Willem. in Usteri Ann. der Bot. 18:26. 1796.
Fourcroea Haw. Syn. Pl. Succ. 73. 1812.
Furcroya Raf. Princ. Somiol. 31. 1814.
Furcroea Benth. & Hook. Gen. Pl. 3:739. 1883.

Plants massive, acaulescent or with short stout trunks; leaves closely crowded, ensiform, very heavily coriaceous, usually armed with recurved thorns upon the margin; inflorescence a panicle, usually massive and many-flowered, the flowers frequently replaced by bulbils; perianth rotate, 6-parted, the tube short and cylindrical, the lobes subequal, spreading, white or greenish; stamens 6, the filaments attached to the throat of the perianth tube; anthers versatile; pistil 3-celled, oblong, containing numerous ovules; style short and thick; stigma capitate; fruit an oblong, loculicidal capsule containing many flat seeds.

1. FURCRAEA CABUYA Trel. in Ann. Jard. Bot. Buitenzorg, II Ser. Suppl. 3:906. *pl. 36.* 1910.

Fourcroya gigantea Vent. and *F. tuberosa* (Miller) Ait. acc. to Seem. Bot. Voy. Herald, 216. 1854.

Fig. 8. *Furcraea cabuya*

Massive xerophytes 2–7 m. tall, somewhat resembling an *Aloe* or a *Yucca,* acaulescent or with a short stout trunk less than 1 m. tall; leaves in a dense rosette, lance-ensiform, gradually acuminate to a sharp acumen, 1–3 m. long, 15–20 cm. broad, very heavily coriaceous, the margin beset with stout recurved thorns, green or somewhat glaucous, the upper surface rather closely lined with paler stripes; panicles very massive, bearing many greenish white flowers, or these replaced by bulbils; flowers very shortly pedicellate; ovary narrowly oblong-fusiform, about 1.5 cm. long; perianth segments barely united at the base, ovate, acuminate, about 1.5–3.0 cm. long.

Costa Rica, Panama, in semi-xerophytic llanos and savannas.

CHIRIQUÍ: Llanos del Volcán, *Seibert 343, Seemann.*

1a. FURCRAEA CABUYA var. INTEGRA Trel. loc. cit. 907. 1910.

Like the preceding, but the leaf margins unarmed.

PANAMÁ: near Panama City, *Verner s. n.*

This magnificent species is the outstanding xerophyte of Panama, where it is popularly known as *Cabuya* or *Cabuya blanca* since the time of the Spanish naturalist Oviedo. The typical and the unarmed varieties are sometimes distinguished as *Cabuya con espinas* or *Cabuya sin espinas.* Standley reports that the flowers

occasionally are employed in flavoring food. According to Seemann, the plant was cultivated "to a considerable extent" as early as 1846 on account of its fibre from the leaves, used for making rope and hammocks. Such cultivation, however, is not apparent at the present time.

2. AGAVE L.

Agave L. Sp. Pl. 323. 1753.

Plants massive, acaulescent or with a short stout trunk; leaves closely crowded, ensiform, heavily coriaceous, usually armed with spines upon the margin, the tip ending in a stout spine; inflorescence a panicle or paniculate spike, usually massive and many-flowered, but the flowers frequently replaced by viviparous bulbils; perianth funnel-shaped, 6-parted, the tube rather elongate, the lobes subequal, usually greenish white; stamens 6, widely exserted from the perianth, the filaments attached to the perianth tube; anthers versatile; pistil 3-celled, containing numerous ovules; style rather short and thick; stigma capitate; fruit a loculicidal capsule containing many seeds.

1. Agave panamana Trel. in Standl. Contr. U. S. Nat. Herb. 23:114. 1920.

Leaves rather thin, 6–7 dm. long, 3–5 cm. wide, with a stout black terminal spine 1–2 cm. long, and rather distant marginal teeth 0.1–0.2 cm. long; panicle 1–3 m. tall with numerous secondary branches 5–12 cm. long, the flowers numerous at the tips of the corymbosely branching secondary peduncles, frequently replaced by viviparous bulbils; flowers funnel-shaped, greenish white or yellow, the narrow tube about 3.5 cm. long, the lobes acuminate, virtually erect, 2.0–2.5 cm. long; stamens widely exserted, the filaments 5–6 cm. long, the anthers sublinear, 2 cm. long.

Known only from Panama on the semi-xerophytic shores and islands of the Gulf of Panama.

panamá: Urava Island, *Howe s. n.*; rocky headlands, Vacamonte Point, *Allen 2958*.

The Agaves include the familiar "Century Plants." *A. picta* Salm-Dyck, with yellow leaf margins, is cultivated occasionally in Panama.

3. BOMAREA Mirb.

By E. P. Killip

Bomarea Mirb. Hist. Nat. Pl. 9:71. 1804.

Vines (the Panama species), often high-climbing, generally with fibrous, often tuberiferous roots and resupinate leaves, the stem unbranched, terminating in an umbel of showy flowers, the rays simple or branched; perianth funnel-shaped, the tube none, the outer segments (sepals) prevailingly oblong or oblanceolate, firm in texture, the inner (petals) unguiculate, thinner than the sepals and subequaling or exceeding them; anthers oblong, basifixed; fruit usually 3-angled and dehiscent, with red, subglobose seeds.

a. Umbel rays usually more than 10, unbranched, ebracteolate or with a small bractlet near base; sepals red or orange, shorter than the petals; plants of the highlands.
  b. Rays and ovary glabrous, the rays very slender ..... 1. B. CHIRIQUINA
  bb. Rays and ovary viscous-tomentulose ..... 2. B. HIRSUTA
aa. Umbel rays usually fewer than 10, furcate or with several divaricate pedicels, bracteolate; sepals pink, subequaling or longer than the petals; plants of the lowlands.
  b. Ovary and rays pubescent, the rays bearing several subsecund, divaricate pedicels ..... 3. B. CHONTALENSIS
  bb. Ovary and rays glabrous, the rays furcate, the branches ascending.
    c. Flowers 4 cm. or more long; bractlets leaf-like, more than 3 cm. long ..... 4. B. ALLENI
    cc. Flowers smaller; bractlets much reduced, not more than 2 cm. long ..... 5. B. EDULIS

1. BOMAREA **chiriquina** Killip, sp. nov.

Caulis volubilis, glaber; folia lanceolata, membranacea, glabra; radii 12–35, tenuissimi, ut ovarium glaberrimi; segmenta perianthii inaequalia, petalis quam sepalis ca. 5 mm. longioribus. (Eubomarea § Caldasianae)

Fig. 9. *Bomarea chiriquina*

Vine, 4–10 m. long, glabrous throughout, even the rays and the ovary without a vestige of indument; stem subangular; leaves lanceolate, 3–15 cm. long, 1–4 cm. wide, attenuate-acuminate at apex, abruptly tapering at base to an undulate-margined petiole up to 1 cm. long, closely nerved, membranous; outer bracts oblanceolate, 2–3 cm. long, 0.5–1 cm. wide, acuminate, reflexed, reddish, the inner narrowly linear, erect or divaricate, about half as long; umbel to 35-rayed, the rays 3–8 cm. long, very slender, wiry, simple, ebracteolate or bearing toward the base a small ovate bractlet up to 3 mm. long; ovary short-turbinate; sepals oblong-spatulate, about 2.5 cm. long and 8 mm. wide, obtuse, scarlet (red, orange, pinkish orange); petals about 3 cm. long, orange, obscurely purple-spotted, the blade cuneiform, 1 cm. wide, gradually tapering to a claw about 1 cm. long; anthers oblong, 3 mm. long, with a circular cavity at the base; fruit 1.5–2 cm. wide, sharply angled, soon dehiscent; seeds 4 mm. long.

Mountains of Chiriquí, alt. 1500–2000 m.

CHIRIQUÍ: Cerro Punta, alt. 2000 m., Jan. 21-24, 1939, *Allen 1556* (U. S. Nat. Herb., TYPE), *Bouché 432;* Río Chiriquí Viejo, *G. White 10, P. White 327;* Volcán de Chiriquí, *Woodson, Allen & Seibert 914, 953, Davidson 941;* between Finca Lérida and Peña Blanca, *Woodson & Schery 296, 297, 327.*

2. Bomarea hirsuta (HBK.) Herb. Amaryl. 114. 1837.

Alstroemeria hirsuta HBK. Nov. Gen. & Sp. 1:285. 1816.

Leaves lanceolate or oblong-lanceolate, 4–10 cm. long, 1–3 cm. wide, cuspidate-acuminate, membranous; umbel rays usually 20–40, up to 5 cm. long, rarely fewer or longer, simple, ebracteolate, viscous-tomentose; ovary turbinate-campanulate, viscous-tomentose; sepals oblong, 1.5–2 cm. long, red or crimson; petals cuneate-unguiculate, 2–3 cm. long, red or orange, unspotted or with numerous very small, reddish or brownish spots.

In typical *B. hirsuta* the under-side of the leaves is densely hirsute or hirsute-tomentose. This is common in Colombia, rare in Costa Rica, and so far not known from Panama. The following variety, with the leaves glabrous, or very sparingly pubescent beneath, has the same range as the typical form and is represented in Panama by several collections:

1a. Bomarea hirsuta var. **concolor** (Cuf.) Killip, comb. nov.

Bomarea Caldasiana Herb. var. *concolor* Cuf. in Archiv. Bot. Sist. Fitog. 9:186. 1933.

Chiriquí: Cuesta de Cerro Quemado, eastern slope of Volcán de Chiriquí, alt. 1800–2160 m., *Maxon 5371;* Bajo Chorro, alt. 1900 m., *Woodson & Schery 615, Davidson 89* (approaching *B. costaricensis* Kränzl.), *338;* Loma Larga to summit, Volcán de Chiriquí, alt. 2500–3380 m., *Woodson, Schery & Seibert 1072;* Casita Alta to Cerro Copete, 2300–3300 m., *Woodson & Schery 343.*

3. Bomarea chontalensis Seemann in Gard. Chron. 1871:479, 1387, *f. 305.* 1871.

Bomarea edulis var. *chontalensis* Baker, Amaryl. 154. 1888.

Stem glabrous; leaves oblong-lanceolate, 8–15 cm. long, 1.5–5 cm. wide, membranous, glabrous; bracts similar and subequal to the leaves; umbel rays 2–6, stout, up to 25 cm. long, ferruginous-puberulent, bearing 3–7 subsecund, pediceled flowers, bracteolate at the base of the divaricate pedicels, the bractlets lance-ovate, about 1.5 cm. long, pubescent; ovary elongate-obconical, ferruginous-tomentulose; sepals broadly obovate, 2.5–3 cm. long, 1–1.5 cm. wide, pink; petals slightly shorter than the sepals, the blade broadly ovate, rounded, pale yellow, greenish toward the apex, blotched with brown.

Nicaragua to western Panama, at low elevations.

Bocas del Toro: Nievacita, near sea-level, *Woodson & Schery 1027.*

4. Bomarea **Alleni** Killip, sp. nov.

Herba volubilis vel subvolubilis, ubique glabra; folia lanceolata, membranacea; bracteae foliaceae; radii 3–5, simplices vel furcati, bracteolis foliaceis, amplis; ovarium longe turbinatum; sepala et petala subaequalia, rosea, sepalis oblanceolatis, petalis oblongo-spathulatis, unguiculatis. (Eubomarea § Edules)

Vine or vine-like herb, 7 m. long, glabrous throughout; leaves lanceolate, 15–20 cm. long, 3–5 cm. wide, attenuate-acuminate at the apex, abruptly nar-

rowed at the base to a stout, margined petiole up to 1.5 cm. long, membranous, the nerves subequally prominent, the cross-veins conspicuous; bracts 6, whorled at the base of the inflorescence, similar to and slightly smaller than the leaves; umbel 3- to 5-rayed, the rays up to 30 cm. long, unbranched or with 1 or 2 short 1-flowered lateral branches, bracteolate, the bractlets similar to the bracts, decreasing from 11 cm. long and 4 cm. wide (lowermost) to 3.5 cm. long and 1.5 cm. wide; ovary long-turbinate, 6-angled, truncate; sepals oblanceolate, 4–5.5 cm. long, 1.3–1.5 cm. wide, minutely corniculate dorsally just below the rounded apex, shell-pink, with a few scattered red spots near the apex within; petals equal to or slightly shorter than the sepals, shell-pink, densely spotted with reddish brown, bearing a green blotch near the apex, the blade oblong-spatulate, 8–14 mm. wide at the widest point, tapering gradually to a claw about 1.5 cm. long; anthers ovate-oblong, 3 mm. long, with a circular cavity at the base; style about 3.5 cm. long; young fruit 3.5 cm. long, 2 cm. in diameter.

PANAMÁ: El Valle de Antón, along the Río Indio trail, alt. 500–700 m., Jan. 31, 1935, *Hunter & Allen 325* (Mo. Bot. Gard., TYPE; duplicate at U. S. Nat. Herb.); vicinity of La Mesa, north of El Valle de Antón, alt. 1000 m., *Allen 2491*.

Related to the Colombian *B. Carderi* Mast., differing in having large, leaf-like bractlets and proportionately broader sepals.

5. BOMAREA EDULIS (Tussac) Herb. Amaryl. 111. 1837.

Alstroemeria edulis Tussac, Fl. Antill. 1:109. *pl. 14.* 1808.

Fig. 10. *Bomarea edulis*

Stem glabrous; leaves lanceolate, 6–10 cm. long, 1.5–3 cm. wide (rarely smaller or larger), membranous, glabrous; bracts similar to the leaves but usually much smaller; umbel 4- to 10 (rarely to 20)-rayed, the rays slender, up to 12 cm. long, once- or twice-forked, bracteolate at the forks, the bractlets linear-lanceolate, up to 1.5 cm. long; sepals obovate or oblong-obovate, 2–3 cm. long, bright pink; petals cuneate-spatulate, subequal to the sepals, yellow, green-tinged and purple-spotted.

Mexico, Central America, and the West Indies; apparently also in eastern Brazil. At low elevations.

COCLÉ: between Las Margaritas and El Valle, *Woodson, Allen & Seibert 1342*.

4. HYPOXIS L.

Hypoxis L. Syst. 986. 1759; Brackett in Rhodora 25:120-147. 1923.

Fabricia Thunb. in Fabricius, Reise Norweg. 23. 1779, in part.
Niobea Willd. ex. Schult. f. Syst. Bot. 7:762. 1830.
Franquevillea Zoll. apud Miq. Fl. Ind. Bat. 3:586. 1858.

Small scapose herbs from corm-like rhizomes, scatteringly pilose throughout, especially in the inflorescence; leaves narrow, grass-like, basal; inflorescence bearing 1 or several small yellow flowers, cymose; perianth segments 6, barely united at the base, without a tube, regular, the outer usually somewhat sepal-like; stamens 6, the filaments united to the base of the perianth; pistil 3-celled, inferior, more or less truncate at the tip, without a beak; fruit a capsule, dehiscing by longitudinal slits.

a. Leaf sheaths at length disintegrating into stiff persistent fibers..... 1. H. HUMILIS
aa. Leaf sheaths wholly disintegrating, not fibrous..... 2. H. DECUMBENS

1. Hypoxis humilis HBK. Nov. Gen. & Sp. 1:286. 1816; Brackett, loc. cit. 144. 1923.

Niobea pratensis Willd. ex Schult. f. Syst. Bot. 7:762. 1830.

Fig. 11. *Hypoxis humilis*

Small scapose herbs; corm globose to subcylindric, 5–11 cm. thick, surrounded by the bristle-like fibers of disintegrated leaves; leaves linear, 0.8–2.8 cm. broad, 6–35 cm. long, rather densely pilose; peduncles 1- to 2-flowered, 1–18 cm. long, slender, pilose; perianth segments narrowly elliptic, 3–5 mm. long; capsules subglobose, 3–6 mm. long.

Mexico to Argentina, in llanos at fairly high elevations.

CHIRIQUÍ: Llanos del Volcán de Chiriquí, *Allen 997*.

2. Hypoxis decumbens L. Pl. Jam. Pugill. 11. 1759; Brackett, loc. cit. 129. 1923.

Hypoxis caricifolia Salisb. Prodr. 248. 1706.
Hypoxis gracilis Lehm. apud Schult. f. Syst. Bot. 7:764. 1830.
Hypoxis decumbens var. *mexicana* (Schult. f.) Jennings in Ann. Carn. Mus. 11:97. 1917.

Small scapose herbs; corm cylindric to ellipsoid, 0.7–2.0 cm. long, the surrounding leaf sheaths membranous, not fibrous; leaves linear to lanceolate, 1–4 dm. long, 2–12 mm. broad, sparsely pilose to glabrate; peduncles 1- to 4-flowered, filiform, 2–20 cm. long; perianth segments lanceolate, 4–10

mm. long; capsule club-shaped, cylindric or slenderly ellipsoid, 0.6–1.7 cm. long.

Mexico to Brazil; Antilles, in meadows, llanos, and savannas.

CHIRIQUÍ: Piedro de Lino, *Killip 3570;* Llanos del Volcán, *Seibert 336.*

5. CURCULIGO Gaertn.

CURCULIGO Gaertn. Fruct. et. Sem. 1:63. *t. 16, f. 11.* 1788.
Aurota Raf. Fl. Tellur. 3:61. 1836.

Scapose herbs from corm-like rhizomes, scatteringly pilose throughout; leaves narrow, grass-like (in Panama), basal; inflorescence mostly 1-flowered; perianth segments yellowish, 6, equal, barely united at the base but appearing to have a long tube because of the narrow beak of the ovary; stamens 6, the filaments united to the base of the perianth; pistil 3-celled, inferior, provided with a long narrow beak; fruit rather fleshy and indehiscent.

Fig. 12
Curculigo scorzoneraefolia

1. CURCULIGO SCORZONERAEFOLIA (Lam.) Baker in Jour. Linn. Soc. Bot. 17:124. 1878; Brackett in Rhodora 25:160. 1923.

Hypoxis scorzoneraefolia Lam. Encyc. Meth. Bot. 3:183. 1789.

Small scapose herbs, scatteringly pilose throughout; leaves linear to lanceolate, 10–35 cm. long, 1.5–14 mm. broad; peduncles mostly 1-flowered, 5.0–8.5 cm. long; perianth segments lanceolate, 0.7–1.4 cm. long, pilose without; ovary (including the narrow beak) 2–4 cm. long.

Mexico to South America; Antilles, savannas and llanos.

PANAMÁ: Pacora, *Woodson, Allen & Seibert 740.*

6. CRINUM L.

CRINUM L. Sp. Pl. 291. 1753.
Tanghekolli Adans. Fam. Pl. 2:57. 1763.
Scadianus Raf. Atl. Jour. 164. 1833.
Liriamus Raf. Fl. Tellur. 4:23. 1836.
Crinopsis Herb. Amaryll. 270. 1837.
Pancratio-Crinum Herb. ex Steud. Nom. 2:250. 1841.

Usually rather massive scapose herbs with tunicated bulbs and basal, usually succulent leaves; inflorescence scapose, umbelliform, bearing few to several showy, sessile or subsessile flowers subtended by 2 or more spathaceous bracts; perianth salverform, with a long slender tube and a spreading limb of 6 more or less equal lobes, white, or more or less deeply flushed with red or purple; stamens 6, inserted at the base of the perianth lobes; filaments terete or subterete, slender; anthers versatile; fruit a rather fleshy, asymmetrical capsule, tardily dehiscent.

a. Leaves lorate or ensiform, greatly elongate, sessile.
  b. Perianth lobes erect or ascending, linear, nearly as long as the tube; flowers with short but distinct pedicels ..... 1. C. LONGIFLORUM
  bb. Perianth lobes reflexed, lanceolate, ½–⅓ as long as the tube; flowers sessile ..... 2. C. ERUBESCENS
aa. Leaves oblong-elliptic, narrowed to a conspicuous subpetiolar base; perianth lobes widely spreading, elliptic-oblong, about ⅓ as long as the tube; flowers shortly pedicellate ..... 3. C. DARIENENSIS

1. Crinum longiflorum Herb. Amaryll. 271. 1837.

Amaryllis longifolia var. *longiflora* Ker in Bot. Reg. *pl. 303.* 1818.

Leaves rather broadly ensiform, narrowly acuminate, 6–9 dm. long, 6–8 cm. broad toward the base; flowering scape stout, about 8 dm. tall, involucral bracts narrowly lance-trigonal, 6–9 cm. long, bearing 4–8 showy white flowers usually deeply flushed with purple in the tube; flowers with stout pedicels 2–3 cm. long; perianth tube 8–9 cm. long, very slender, the lobes linear, about 9 cm. long, erect or ascending; stamens widely exserted, the filaments red.

Very widely cultivated, considered by Dean Herbert to be a native of Jamaica and Antigua.

BOCAS DEL TORO: Little Bocas, *von Wedel 2531;* Western River, *von Wedel 2789a.*

Widely known as *Lirio* in Panama. The specimens cited above probably are escapes.

2. Crinum erubescens Ait. Hort. Kew. 1:413. 1789.

Crinum Commelini Jacq. Hort. Shoen. 2:40. *pl. 202.* 1798.

Crinum Kunthianum M. Roem. Fam. Nat. Syn. 4:80. 1847.

Fig. 13. *Crinum darienensis*

Bulb ovoid, 7–10 cm. in diameter; leaves basal, numerous, lorate, gradually acuminate, 3–5 dm. long, 5–7 cm. broad; flowering scape stout, 3–4 dm. long, involucral bracts lance-trigonal, 6–8 cm. long, bearing 4–12 flowers; flowers white, usually deeply tinged with purple without, sessile; perianth tube 15–20 cm. long, very slender, the lobes lanceolate, 7–8 cm. long; stamens greatly exserted, the filaments scarlet or purple.

Widespread in tropical America, and frequently cultivated. In Panama apparently spontaneous in wet soil beside streams at rather low elevations.

BOCAS DEL TORO: Río Cricamola, *Woodson, Allen & Seibert 1910.* CANAL ZONE: Barro Colorado Island, *Seibert 567.*

Popularly known as *Lirio.*

3. CRINUM DARIENENSIS Woodson in Ann. Missouri Bot. Gard. 25:824. 1938.

Bulbs subcylindrical, 12–13 cm. long, 2–3 cm. thick, densely tunicated; leaves basal, oblong-elliptic, acute to shortly acuminate, gradually produced into a conspicuous subpetiolar base, 20–30 cm. long, the blade 4–6 cm. broad; flowering scape 15–20 cm. long, relatively slender, the involucral bracts ovate-trigonal, 5–7 cm. long, 3- to 5-flowered; flowers white, or very faintly tinged with pink; pedicels 0.5 cm. long or scarcely manifest; perianth tube very slender, 15–20 cm. long, the lobes lanceolate to oblong-elliptic, 5–6 cm. long, spreading; stamens widely exserted, the filaments purple.

Panama, in humid forest.

COCLÉ: El Valle de Antón, *Allen 1659.* DARIÉN: between Pinogana and Yaviza, *Allen 264.*

7. ZEPHYRANTHES Herb.

ZEPHYRANTHES Herb. App. Bot. Reg. 7:36. 1821; Herb. Amaryll. 170. 1837; Baker, Handb. Amaryll. 30. 1888.
Pyrolirion Herb. App. Bot. Reg. 7:37. 1821.
Habranthus Herb. in Bot. Mag. *pl. 2464.* 1824.
Mesochloa Raf. Fl. Tellur. 4:10. 1836.
Plectronema Raf. loc. cit. 1836.
Pogonema Raf. loc. cit. 1836.
Argyropsis M. Roem. Fam. Nat. Syn. 4:125. 1847.
Arviela Salisb. Gen. Pl. Fragm. 135. 1866.
Atamosco Greene, Pittonia 3:187. 1897.

Delicate, glabrous, acaulescent herbs arising from underground bulbs; leaves linear, contemporary with the flower; scape 1-flowered, consisting of an elongate peduncle bearing terminally a 1-valved spathe from which arises the solitary pedicel; perianth funnel-shaped with a short tube and subequal lobes, stamens 6, glabrous; filaments filiform, inserted in the throat or at the base of the perianth tube; anthers linear, versatile, attached below the middle; style slender, from ½ as long to almost as long as the perianth; stigma trifid or trilobed; capsule ovate, 3-valved, many seeded.

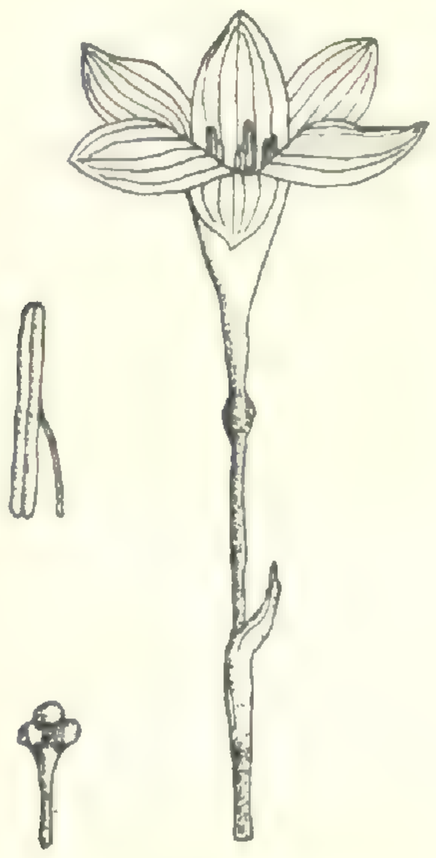

Fig. 14
Zephyranthes citrina

a. Flowers yellow; stigma subcapitate; perianth tube manifest; filaments inserted at top of perianth tube ..... 1. Z. CITRINA
aa. Flowers pink or white; stigma trifid; perianth tube almost obsolete; filaments inserted near base of perianth.
  b. Flowers pink, short, about 3 cm. long; petals obtuse; leaves usually one pair, no longer than the peduncle ..... 2. Z. ROSEA
  bb. Flowers white, about 3.5 cm. long; petals acute; leaves usually 4, often longer than the scape ..... 3. Z. TUBISPATHA

1. ZEPHYRANTHES CITRINA Baker in Bot. Mag. *pl. 6605.* 1882.

64339 6/7/46

Z. Eggersiana Urban, Symb. Ant. 5:292. 1907.

Leaves linear, up to 35 cm. long and about 2 mm. wide; peduncle thicker than in the other two species, about 16 cm. long; spathe about 2 cm. long, its tube about 8 mm. long, attenuate on one side into an uncleft tip; pedicel 2–3 cm. long; perianth yellow, the tube manifest, almost 1 cm. long, the lobes elliptic-obovate, about 2.5 cm. long and 8 mm. wide, obtuse apically; filaments inserted at the top of the perianth tube, about 12 mm. long; style about 16 mm. long, bearing a stout 3-lobed, subcapitate stigma.

Vaguely referred to as growing in tropical America; reported from Cuba and Trinidad. The type supposedly came from British Guiana. The plant is now cultivated in Florida and perhaps elsewhere. In Panama the species has been collected only from "Maccaw Hills" on the inhabited Columbus Island where it was growing in association with the other known Panamanian species, and may possibly have been introduced.

BOCAS DEL TORO: Isla Colón, alt. 0–120 m., *von Wedel 543.*

2. ZEPHYRANTHES ROSEA Lindl. in Bot. Reg. *pl. 821.* 1824; Herb. in Bot. Mag. *pl. 2537.* 1825.

Amaryllis rosea Spreng. Syst. 4. Cur. Post. 133. 1827.
A. carnea Schult. f. Syst. Bot. 7:799. 1830.

Leaves linear, up to 12 cm. long, about 3 mm. wide; peduncle about 10 cm. long, bearing at its apex a spathe about 17 mm. long; spathe tube cylindric, about 1 cm. long, attenuate on one side into a deeply cleft tip; pedicel slender, up to 3 cm. long; perianth pink, the tube very short, less than 5 mm. long, the lobes obovate or oblanceolate, almost 3 cm. long, 6–8 mm. wide, obtuse apically; filaments inserted at the base of the tube, about 14 mm. long; anthers linear, versatile, attached below the middle, about 6 mm. long; style slender, up to 2.5 cm. long, bearing a trifid stigma; stigma lobes linear, about 2 mm. long.

Zephyranthes bifolia M. Roem. (Fam. Nat. Syn. 4:125. 1847) was for many years considered as a synonym for this species, but has recently been reinstated as a valid species by Hume (Bull. Torrey Bot. Club 62:405. 1935).

Known from the West Indies and originally described from a Cuban specimen; probably occurring generally in the American tropics although, as with other species of this genus, it is difficult to know whether the plant is native or introduced in a given region.

BOCAS DEL TORO: Isla Colón, alt. 0–120 m., *von Wedel 544.*

3. ZEPHYRANTHES TUBISPATHA Herb. App. Bot. Reg. 7:96. 1821.

Amaryllis tubispatha Gawl. in Bot. Mag. *pl. 1586.* 1813.
Amaryllis nervosa HBK. Nov. Gen. & Sp. 1:278. 1816.
?Zephyranthes Mesochloa Herb. in Bot. Reg. *pl. 1345, 1361.* 1830.
Zephyranthes nervosa Herb. Amaryll. 172. 1837.
Zephyranthes Lindleyana Herb. loc. cit. 174. *pl. 35. fig. 5.* 1837.

Leaves linear, up to 18 cm. long, about 4 mm. wide; peduncle about 12 cm. long, bearing at its apex a spathe about 2 cm. long; spathe tube cylindric, about

1 cm. long, attenuate on one side into a deeply cleft tip; pedicel slender, up to 3.5 cm. long; perianth white, the tube very short, less than 5 mm. long, the lobes obovate or oblanceolate, almost 3.5 cm. long, 6–8 mm. wide, acute apically; filaments inserted at the base of the tube, about 17 mm. long; anthers linear, versatile, attached below the middle, about 6 mm. long; style slender, up to 2.5 cm. long, bearing a trifid stigma; stigma lobes linear, about 2 mm. long.

Known from Argentina (?) and Jamaica (fide Bot. Mag. loc. cit.); specimens of probably this species are known from Tobago and northern Central America. Like the other two species, this plant has been introduced into Florida and probably elsewhere.

BOCAS DEL TORO: Isla Colón, alt. 0–120 m., *von Wedel.*

This Wedel plant appears to be but a variety or form of the preceding species, in association with which it was found growing. However, the plant matches the illustration of Z. *tubispatha* in the Bot. Mag. almost perfectly, but has slightly smaller dimensions than those given for the species by Baker in his 'Handbook of Amaryllideae.' It seems best to consider the Wedel specimen as Z. *tubispatha,* distinct from Z. *rosea,* until further collections can settle its position definitely.

8. EUCHARIS Planch.

EUCHARIS Planch. ex Linden, Cat. 8:3. 1853.

Mathieua Klotzsch in Otto & Dietr. Allg. Gartenzeit. 21:337. 1853.

Moderate-sized scapose herbs with tunicated bulbs and broadly laminate, narrowly petiolate basal leaves; inflorescence scapose, umbelliform, involucrate, bearing few to several showy pedicellate flowers; perianth infundibuliform, with a narrowly funnel-shaped tube and a spreading limb of 6 broad ovate or ovate-lanceolate segments; stamens inserted at the throat of the perianth tube, shorter than the lobes; filaments broadly dilated and petaloid at the base, forming a conspicuous corona of free or united segments; anthers versatile; pistil inferior, 3-celled; fruit a tardily dehiscent, somewhat fleshy capsule containing a few large ovoid seeds.

Fig. 15. *Eucharis candida*

a. Perianth about 10–11 cm. long, the limb about 9–10 cm. broad; corona segments with lateral auricles ..... 1. E. CANDIDA

aa. Perianth about 6–7 cm. long, the limb about 4.5–5.0 cm. broad; corona segments triangular-dentiform, without lateral auricles ..... 2. E. BOUCHEI

1. EUCHARIS CANDIDA Planch. in Fl. Serres *pl. 788.* 1853.

Bulbs ovoid, 3.5–4.0 cm. long or more; leaf-blades broadly oval, shortly and abruptly acuminate, 9–15 cm. long, 9–10 cm. broad, obtusely narrowed to a slender petiole 15–20 cm. long; flowering scape 3–5 dm. long, rather stout, subcompressed, bearing 6–10 handsome white flowers at the tip; perianth tube 6–7 cm. long, about 0.1 cm. in diameter at the base, dilating abruptly to a conical throat about 1 cm. broad, the lobes broadly ovate, obtuse, 4–5 cm. long; corona erect, about 1 cm. tall, composed of 5 laterally auriculate segments united for about half their length, terminated by the filiform staminal filaments about 1 cm. long.

Colombia and Panama, in highland forests. Frequently cultivated and possibly an escape in Panama.

COCLÉ: El Valle de Antón, *Hunter & Allen 338.*

An extremely handsome Amaryllid popularly known as *Eucaristo* and *Eucharistia.*

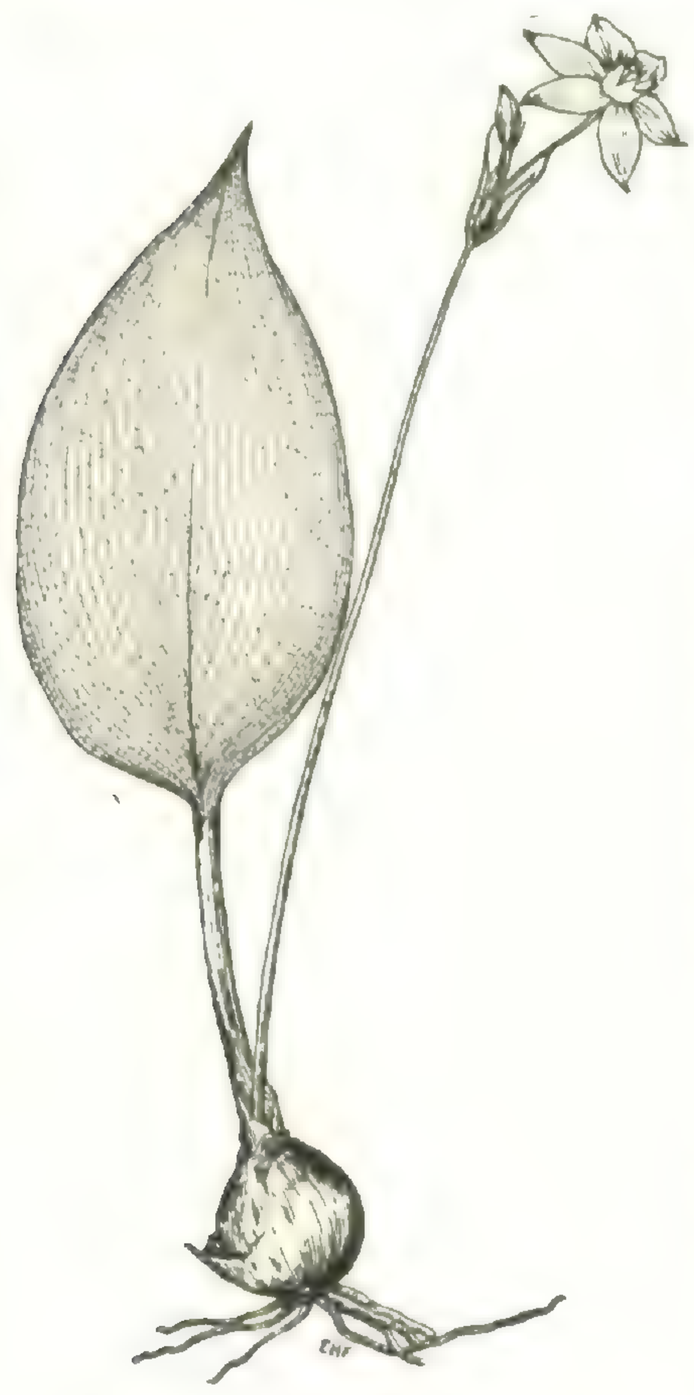

Fig. 16. *Eucharis Bouchei*

2. EUCHARIS BOUCHEI Woodson & Allen in Ann. Missouri Bot. Gard. 24:181. 1937.

Bulbs ovoid, about 4 cm. long; leaf-blades broadly oval to ovate-lanceolate, abruptly acuminate, 20–35 cm. long, 11–15 cm. broad, rather abruptly narrowed to the slender petiole 20–30 cm. long; flowering scape 30–50 cm. long, bearing 4–8 rather showy white flowers at the tip; perianth tube 3.0–4.5 cm. long, about 0.1 cm. wide at the base, abruptly widened to a conical throat about 0.5–0.7 cm. in diameter, the lobes ovate to oblong-ovate, acute to obtuse, 2.0–2.5 cm. long; corona erect, about 0.8 cm. tall, composed of 5 acutely tapered segments united for about half their length.

Panama, in highland forest. Perhaps the same species is represented in Costa Rica by *E. himeroessa* Sandw. *nom. nud.* apud Standl. Fl. Costa Rica 1:176. 1937.

COCLÉ: El Valle de Antón, *Allen 120, 1228, 2063, Seibert 466.*

9. PANCRATIUM L.

PANCRATIUM L. Sp. Pl. 290. 1753.
Hymenocallis Salisb. in Trans. Hort. Soc. Lond. 1:338. 1812.
Nemepiodon Raf. Fl. Tellur. 4:22. 1836.
Siphotoma Raf. loc. cit. 1836.
Tomodon Raf. loc. cit. 1836.
Choretis Herb. Amaryll. 219. *t. 35.* 1837.
Hymenocalyx Herb. ex Houll. in Rev. Hortic. 40:418. 1869.

Moderately large scapose herbs with tunicated bulbs and rather fleshy basal leaves; inflorescence scapose, umbelliform, involucrate, bearing few to several showy sessile flowers; perianth salverform, with a very narrow tube and six spreading narrowly linear lobes; stamens inserted on the margin of a very conspicuous turbinate corona, the free portion of the filaments long and very slender; anthers linear, versatile; ovary inferior, 3-celled; fruit a fairly large, tardily dehiscent capsule.

1. PANCRATIUM LITTORALE Jacq. Select. Stirp. Amer. Hist. 99. *t. 179, fig. 94.* 1766.

Hymenocallis littoralis (Jacq.) Salisb. in Trans. Hort. Soc. Lond. 1:338. 1812.
Pancratium americanum Mill. Gard. Dict. ed. 8. no. 7. 1768.
Hymenocallis Dryandri M. Roem. Fam. Nat. Syn. 4:175. 1847.
Hymenocallis disticha Herb. App. Bot. Reg. 7:44. 1821.
Hymenocallis acutifolia M. Roem. loc. cit. 174. 1847.
Hymenocallis Staplesiana M. Roem. loc. cit. 175. 1847.
Hymenocallis americana (L.) Salisb. ex Standl. Fl. Pan. Canal Zone, 115. 1928, *sphalm.*

Fig. 17. *Pancratium littorale*

Bulbs ovoid, 5–8 cm. long; leaves lorate, sessile, gradually acute to obtuse, 6–8 dm. long, 2–3 cm. broad, basal; flowering scape compressed, 3–6 dm. long, bearing numerous showy white flowers at the tip; perianth tube 15–20 cm. long, about 0.15 cm. thick, the lobes narrowly linear, 9–12 cm. long, reflexed; corona broadly funnel-shaped, 2.0–2.5 cm. deep, about 3–3.5 cm. in diameter at the margin; staminal filaments very slender, 4.5–5.5 cm. long; style about equalling the anthers.

Widely distributed from the southeastern United States southward throughout the Antilles and tropical America generally; very common on sea beaches and coastal marshes.

BOCAS DEL TORO: Water Valley, *von Wedel 1415.* PANAMÁ: Trapiche Island, Bay of Panama, *Allen 2625.*

Pancratium is the "Spider Lily" of the southeastern United States.

VELLOZIACEAE

1. VELLOZIA Vand.

VELLOZIA Vand. Fl. Lusit. & Bras. Sp. 32. *t. 2.* 1788.
Vellosia Spreng. Syst. 3:338. 1826, *sphalm.*
Vellozoa Lem. Jard. Fleur. 4: *t. 390.* 1853.

Shrubby plants with rather stout, dichotomously branched stems covered with the persistent bases of fallen leaves; leaves crowded at the tips of the branches, stiff and narrow; flowers solitary on long peduncles in the axils of the upper leaves, perfect, regular, sometimes rather showy; perianth segments 6, free, the outer occasionally somewhat calycine; stamens 6 or more; filaments united to the base of the perianth segments; anthers basifixed, longitudinally dehiscent; pistil inferior, 3-celled, the style slender, with a capitate or 3-lobed stigma; fruit a loculicidal capsule; seeds numerous, borne on stalked axile placentas.

1. Vellozia panamensis Standl. in Jour. Wash. Acad. Sci. 15:457. 1925.

Leaves linear, 8–25 cm. long, 0.5–0.9 cm. broad, subulate-attenuate, glabrous above, white-pilose beneath, the margins cartilaginous, smooth; flowering scapes 1–2, stout, 4–6 cm. high, densely covered with stipitate glands; perianth tube nearly 5 cm. long, densely covered with stipitate glands, the lobes linear, about 1 cm. long, glandular without; stamens 12; style filiform, twisted above, exceeding the perianth; capsule 1.5 cm. long, subglobose, densely covered with stout, dark, stipitate glands.

Panama, in highland forest.

chiriquí: Cerro Vaca, *Pittier 5352*.

DIOSCOREACEAE[1]

By C. V. Morton

1. DIOSCOREA L.

Dioscorea L. Sp. Pl. 1032. 1753; C. V. Morton, Carn. Inst. Wash. Publ. 461:241-253. 1936.

Twining vines; leaves simple, alternate or opposite, petiolate, usually cordate at base, acuminate, entire or lobate, glabrous or pubescent with simple hairs, not tendril-bearing, palmately veined, the secondary veins reticulate; flowers regular, unisexual (dioecious); perianth segments 6, similar, equal, ovate to linear-oblong, connate at base; stamens 6, or 3 (rarely alternating with 3 staminodia), borne on perianth segments or on a central disk, free or connate; anthers small, free, opening by longitudinal slits, introrse or extrorse; rudimentary ovary present or absent in the staminate flowers; pistillate flowers with or without minute staminodia; ovary inferior, linear or oblong, 3-celled; ovules 2 in each cell, superposed, pendulous, anatropous; styles 3, short; fruit a loculicidally dehiscent, 3-valved capsule; seed winged at base or all around.

The cultivated yams, often called *ñame* in tropical America, belong to this genus. The following cultivated species, and perhaps others, may be naturalized to some extent in Panama: *Dioscorea alata* L., *D. bulbifera* L., and *D. cayennensis* Lam. Since the cultivated plants are usually sterile, these species have been placed

[1] Published by permission of the Secretary of the Smithsonian Institution.

in the alternative key based on sterile specimens. No descriptions of them are provided.

A specimen of an additional native species, probably undescribed, has been collected near Cana, but the material at hand is in fruit only.

a. Fertile stamens 6.
  b. Leaf blades 3- to 5-lobed; stems and petioles winged; rhachis and perianth segments pubescent; stems twining to the left ..... 7. D. TRIFIDA
 bb. Leaf blades entire.
  c. Rhachis, perianth segments and pedicels pubescent; leaves alternate, not pellucid-lineolate.
   d. Leaf blades glabrous beneath, ovate-lanceolate; stems twining to the right; filaments central, partly connate; anthers opening outwardly ..... 1. D. SAPINDOIDES
   dd. Leaf blades densely soft-pilose beneath, suborbicular; stems twining to the left; filaments borne on the perianth segments, free from each other; anthers opening inwardly ..... 8. D. CYMOSULA
  cc. Rhachis, perianth segments and pedicels glabrous; leaves and stems glabrous.
   d. Leaves partly opposite or subopposite, conspicuously pellucid-lineolate by transmitted light; stems bearing spines at base of the larger leaves; stems twining to the left; flowers solitary, sessile; anthers opening inwardly ..... 2. D. UROPHYLLA
   dd. Leaves alternate, not pellucid-lineolate; stems unarmed, twining to the right; flowers not solitary, or if so, not sessile; anthers opening outwardly.
    e. Filaments slender (0.5 mm. long); leaf blades small (not over 4 cm. long), merely rounded at base, 5-nerved ..... 5. D. PANAMENSIS
    ee. Filaments obsolete; leaf blades larger, cordate at base, 9-nerved ..... 9. D. MACROSTACHYA
aa. Fertile stamens 3; plants wholly glabrous; leaves alternate; stems unarmed.
  b. Anther cells separated by a broad connective; staminodia 3, bifid; flowers sessile, fasciculate ..... 10. D. POLYGONOIDES
 bb. Anther cells contiguous; staminodia none; flowers pedicellate, solitary or rarely in clusters.
  c. Seeds winged at base only.
   d. Filaments very short, inserted on a fleshy, central disk; perianth purple; flowers solitary or clustered ..... 6. D. RACEMOSA
   dd. Filaments obvious, conspicuously enlarged and connate at base; perianth green; flowers always solitary.
    e. Capsules acute ..... 4. D. LEPIDA
    ee. Capsules rounded at apex ..... 3. D. CONVOLVULACEA
  cc. Seeds winged all around; staminate flowers borne in stalked clusters along rhachis ..... 11. D. STANDLEYI

ALTERNATIVE KEY BASED ON STERILE OR FRUITING PLANTS

a. Stems and petioles winged.
  b. Leaf blades deeply 3- to 5-lobed; rhachis and perianth segments pubescent; stems twining to the left ..... 7. D. TRIFIDA
 bb. Leaf blades not lobed; rhachis and perianth segments glabrous; stems twining to the right ..... D. ALATA
aa. Stems and petioles not winged; leaf blades entire.
  b. Leaves opposite, at least in part; stems spiny at base of the larger leaves; leaves pellucid-lineolate.
   c. Stems twining to the left; capsule valves woody ..... 2. D. UROPHYLLA
   cc. Stems twining to the right ..... D. CAYENNENSIS
  bb. Leaves alternate; stems unarmed; leaves not pellucid-lineolate.
   c. Rhachises and ovaries densely pubescent; stems and petioles more or less pubescent.
    d. Stems twining to the right; leaf blades glabrous beneath, ovate-lanceolate ..... 1. D. SAPINDOIDES

dd. Stems twining to the left; leaf blades densely soft-pilose beneath, suborbicular ..... 8. D. CYMOSULA
cc. Rhachises and ovaries glabrous or merely scabrous; leaves and stems glabrous.
d. Leaf blades small (not over 4 cm. long), lanceolate or ovate-lanceolate, rounded at base, 5-nerved; stems twining to the right ..... 5. D. PANAMENSIS
dd. Leaf blades larger, broadly ovate or suborbicular, cordate at base, 9- to 11-nerved.
e. Seeds winged at base only.
f. Stems twining to the left ..... D. BULBIFERA
ff. Stems twining to the right.
g. Capsules acute ..... 4. D. LEPIDA
gg. Capsules rounded at apex ..... 3. D. CONVOLVULACEA
ggg. Capsules unknown ..... 6. D. RACEMOSA
ee. Seeds winged all around.
f. Stems twining to the left; leaf blades abruptly short-acuminate; capsules about 1.7 cm. long ..... 10. D. POLYGONOIDES
ff. Stems twining to the right.
g. Capsules 2.5–3 cm. long; stigmas subsessile ..... 9. D. MACROSTACHYA
gg. Capsules about 1.5 cm. long; stigmas borne on style 1 mm. long ..... 11. D. STANDLEYI

1. DIOSCOREA SAPINDOIDES Presl, Rel. Haenk. 1:33. 1830.

Dioscorea costaricensis Knuth, in Notizbl. Bot. Gart. Berlin 7:189. 1917.
Dioscorea pilosiuscula var. *panamensis* Knuth, in Engl. Pflanzenr. IV. 43:66. 1924.

Stems slender, dextrorsely climbing, sparingly pilosulous, glabrate in age; leaves alternate; petiole up to 8 cm. long, sparingly pilosulous; larger leaf blades ovate-lanceolate, up to 19 cm. long and 9.5 cm. wide, long-acuminate, deeply cordate at base, the central part 5-nerved, the basal lobes each with 4 nerves, glabrous on both sides, the texture thin, the smaller leaf blades rather similar but relatively much narrower; staminate inflorescences 1–3 in an axil, the rhachis 8–30 cm. long, simple or rarely branched, sparsely pilosulous, very slender, the flowers borne in numerous short racemes 6–7 mm. long, these 3- to 8-flowered; pedicels filiform, 2–4 mm. long, densely pilosulous, each bearing a small, brownish bract at base; perianth segments narrowly lanceolate, erect, 1.5–1.8 mm. long, densely pilosulous externally; stamens 6, all fertile, the filaments connate for 0.5 mm. into tube, the free parts 0.5

Fig. 18. *Dioscorea sapindoides*

mm. long, recurved; anthers about 0.25 mm. wide, extrorse; pistillate spikes elongate, 20 cm. long or more, solitary or paired in the axils, the rhachis pilosulous; ovaries and perianth segments pilosulous; fruits oblong, flat, 2 cm. long and 1.1 cm. wide, glabrate at maturity.

Mexico, Costa Rica and Panama.

CANAL ZONE: Quebrada Salamanca, *Dodge, Steyermark & Allen 16992;* Gamboa, *Pittier 4803;* Chepo, *Pittier 4463, 4514* (TYPE of *D. pilosiuscula* var. *panamensis*); Ancón Hill, *Killip 12114;* Empire Railway Station, *Hayes 303, 321* (both teste Knuth). CHIRIQUÍ: Cerro de la Plata, near San Felix, *Pittier 5170;* PANAMÁ: Bejuco, *Allen 984;* Las Sabanas, *Heriberto 199;* La Chorrera, *Paul 503.*

2. DIOSCOREA UROPHYLLA Hemsl. Biol. Centr.-Amer. Bot. 3:361. 1884.

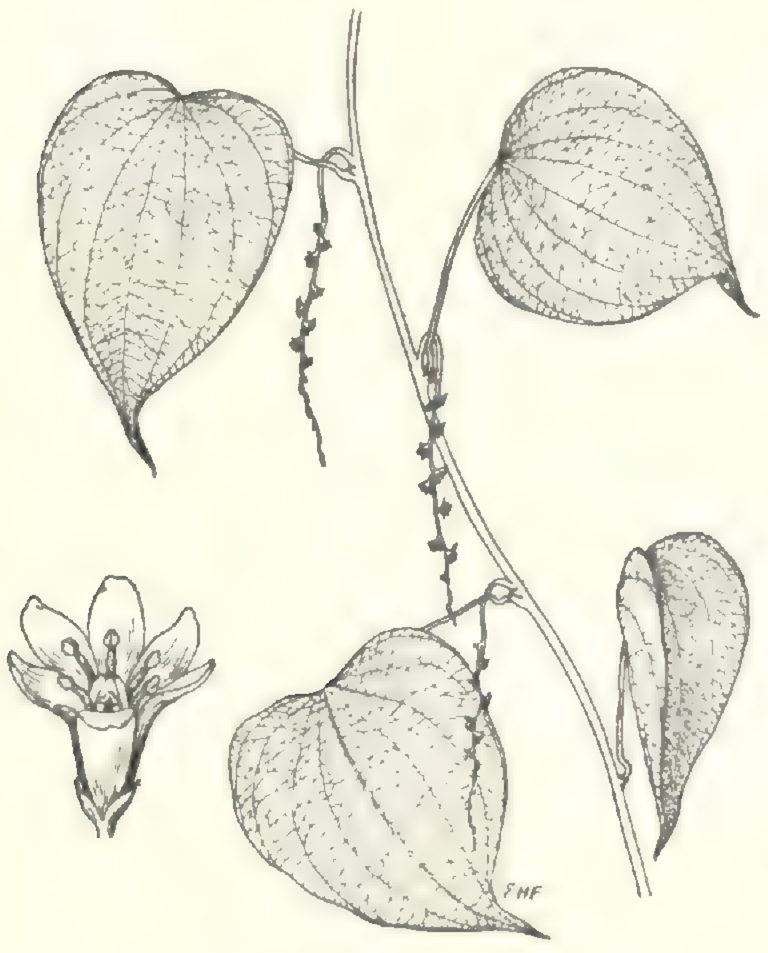

Fig. 19. *Dioscorea urophylla*

Stems climbing sinistrorsely, glabrous, bearing spines at base of larger leaves; leaves often opposite or subopposite; petioles elongate, up to 8 cm. long, often nearly as long as blade, glabrous; leaf blades ovate, up to 9 cm. long and 7 cm. broad, acuminate, truncate at base, 9-nerved, glabrous, membranous, entire, conspicuously pellucid-lineolate; staminate spikes 8–20 cm. long, 1–3 in an axil, unbranched or conspicuously branched, the rhachis glabrous; flowers solitary, sessile; perianth segments about 3 mm. long, connate at base about 0.7 mm., fleshy, glabrous; stamens 6, all fertile, inserted on perianth lobes, the filaments about 0.6 mm. long, the anthers about 0.5 mm. long, introrse; rudimentary ovary large; pistillate spikes solitary, axillary, 10–20 cm. long; capsules oblong, 23–30 mm. long, 13–16 mm. wide, coriaceous, tuberculate, especially near middle of valves; seeds about 2 cm. long (including wing) and 6 mm. wide, winged on lower end.

Confined to Panama.

CANAL ZONE: Fort Kobe Road, *Woodson, Allen & Seibert 1424;* Miraflores Lake, *G. White 172;* Gamboa, *Pittier 3705;* Río Chagres between Río Pequení and Río Indio, *Steyermark & Allen 16777a;* Las Sabanas, *Pittier 6680;* Ancón Hill, *Seibert 396, Greenman & Greenman 5130;* Corozal, *Standley 27337;* between Corozal and Ancón, *Pittier 6772.* COCLÉ: between Aguadulce and Antón, *Woodson, Allen & Seibert 1204.* PANAMÁ: Panama City, *Riley 144, Macbride 2611;* Sabana de Panamá, *Gervais 154;* Punta Paitilla, *Standley 26232;* Río Tapia, Juan Díaz Region, *Maxon & Harvey 6697.*

As pointed out by me (Carn. Inst. Publ. 461:248. 1926), *D. urophylla* belongs to subgenus Helmia, sect. Chondrocarpa, and not to Eudioscorea, sect. Macrogynodium, where it was placed by Knuth. It is undoubtedly the same as the plant reported from Panama by Knuth (Engl. Pflanzenr. IV. 43:84. 1924) as *D. samydea* var. *corcovadensis* Uline. *D. urophylla* is surely very close to the Brazilian *D. samydea* Griseb., and may be conspecific. The type is *Hayes 190* from Panama.

3. DIOSCOREA CONVOLVULACEA Schlecht. & Cham. var. GLABRA (Hemsl.) Uline ex Knuth, in Engl. Pflanzenr. IV. 43:99. 1924.

Dioscorea capillaris var. *glabra* Hemsl. Biol. Centr.-Amer. Bot. 3:354. 1884.

Stems very slender, climbing dextrorsely, glabrous, not spiny; leaves alternate; petioles 1.5–4 cm. long, glabrous; leaf blades ovate, up to 14 cm. long and 13 cm. wide (the upper much smaller), acuminate, entire, deeply cordate at base, usually 11-nerved, glabrous; staminate racemes 1–3 in an axil, unbranched, usually about 10 cm. long, the rhachis slender, glabrous; flowers solitary, pedicellate, the pedicels 2–3 mm. long, glabrous; perianth segments oblong, about 1.8 mm. long, reflexed, free, glabrous; stamens 3, inserted on base of perianth segments, the filaments about 1 mm. long, much enlarged at base and connate with each other, the anthers minute, upwardly dehiscent; staminodia none; rudimentary ovary none; pistillate spikes simple, usually 10 cm. long or less; capsules oblong, rounded at apex, about 18 mm. long and 8 mm. wide, glabrous; seeds about 6.5 mm. long (including wing) and 2.2 mm. wide, winged at basal end.

Mexico to Panama. Trinidad (teste Knuth).

COCLÉ: vicinity of El Valle, *Allen 739*.

Fig. 20
Dioscorea lepida

4. DIOSCOREA LEPIDA Morton in Carn. Inst. Publ. 461:248. 1926.

Stems climbing dextrorsely, glabrous, unarmed; leaves alternate; petioles elongate; leaf blades ovate, up to 12 cm. long and 7.3 cm. wide, long-acuminate, cordate at base, glabrous, membranous, 9-nerved; staminate flowers unknown; capsules green, narrowly oblong, 10–16 mm. long, 5–7 mm. wide, acute, glabrous, soon dehiscent; seeds 7–8 mm. long (including wing), 1.5 mm. wide, winged at base.

Costa Rica and Panama.

COCLÉ: Bismarck, above Penonomé, *R. S. Williams 593;* vicinity of El Valle, *Allen 235*.

It is possible that the single staminate collection referred above to *D. convolvulacea* var. *glabra* is really *D. lepida*, in which case these two species are indistinguishable except by fruiting plants.

5. DIOSCOREA PANAMENSIS Knuth, in Engl. Pflanzenr. IV. 43:109. 1924.

Stems slender and delicate, unarmed, climbing dextrorsely, glabrous; leaves alternate; petioles 0.5–1 cm. long, glabrous; leaf blades lanceolate or ovate-lanceolate, very small, up to 4 cm. long and 2 cm. wide, acuminate, rounded at base, entire, membranous, glabrous, 5-nerved; staminate racemes 1 or 2 in an axil, up to 15 cm. long, unbranched, the rhachis glabrous; flowers solitary or in short, few-flowered racemes, pedicellate,

the pedicels 1.5–2.5 mm. long, glabrous; perianth segments lanceolate, about 1.5 mm. long, connate at base, reflexed; stamens 6, all fertile, central; filaments slender, erect, free, about 0.5 mm. long; anthers minute, about 0.2 mm. long, extrorse; pistillate flowers and capsules unknown.

Known only from Panama.

CANAL ZONE: Ancón Hill, *Killip 3050* (TYPE); Fort Kobe, *Allen 2024*. PANAMÁ: La Chorrera, *Paul 505*.

6. DIOSCOREA RACEMOSA (Klotzsch) Uline in Engler Bot. Jahrb. 22:430. 1897. *Helmia racemosa* Klotzsch in Otto & Dietr. Allg. Gartenzeit. 19:393. 1851.

Stems climbing dextrorsely, unarmed, glabrous; leaves alternate; petiole about 6 cm. long, glabrous; leaf blades broadly ovate, up to 11.2 cm. long and 9.8 cm. wide, acuminate, lightly cordate at base, membranous, entire, glabrous, 9- to 11-nerved; staminate inflorescences 2 or 3 in an axil, up to 17 cm. long, unbranched; flowers solitary or paired or rarely in threes, pedicellate, the pedicels 1–1.5 mm. long; perianth segments purple, ovate-oblong, 1.75 mm. long, spreading, glabrous; stamens 3; staminodia none; filaments very short, distinct, inserted on a fleshy, central disk, the anthers small, upwardly dehiscent; rudimentary style none; pistillate flowers and capsules unknown.

Costa Rica and Panama.

BOCAS DEL TORO: Fish Creek, *von Wedel 2275*. COCLÉ: vicinity of El Valle, *Allen 1258*. PANAMÁ: Campana, *Allen 1869*.

Dioscorea borealis Morton, of Costa Rica, was originally described (Jour. Wash. Acad. Sci. 27:304. 1937) as belonging to the section Centrostemon. This was due to erroneous observation of the androecium. It really belongs in section Cycladenium, and is very close to *D. racemosa* var. *Hoffmannii* Uline from description. This variety may be specifically distinct from typical *D. racemosa*, the type of which was collected by Warscewicz in "Central America."

7. DIOSCOREA TRIFIDA L. f. Suppl. 427. 1781.

Stems climbing sinistrorsely, glabrous, at least the lower conspicuously 4-winged on the angles; leaves alternate; petioles up to 15 cm. long, winged, minutely puberulous; leaf blades variable, the larger leaves deeply 5-lobed, over 15 cm. long and wide, the smaller leaves deeply 3-lobed (the lobes all acute), deeply cordate at base, pellucid-lineolate, minutely puberulous on upper surface and along veins beneath; staminate spikes 2–5 in an axil, shorter than leaves, unbranched, the rhachis subtomentose; flowers solitary (or subfasciculate?), short-pedicellate, the pedicels 1.5–2 mm. long, pilosulous; perianth segments oblong, about 2.5 mm. long, pilosulous externally; stamens 6, all fertile, borne on perianth segments, the filaments about 1 mm. long, the anthers 0.25 mm. long, introrse; rudimentary style conspicuous; ovary pilose; capsule 27 mm. long and 17 mm. wide (teste Knuth), puberulent.

Guatemala, south to Peru and Brazil. West Indies.

CANAL ZONE: Balboa, *Standley 26453;* between France Field and Catival, *Standley 30396*. PANAMÁ: Río Tapia, *Standley 28120*.

8. Dioscorea cymosula Hemsl. Biol. Centr.-Am. Bot. 3:355. 1884.

Dioscorea cymosula var. *Duchassaingii* Uline ex Knuth, in Notizbl. Bot. Gart. Berlin 7:203. 1917.

Dioscorea permollis Knuth, in Engl. Pflanzenr. IV. 43:61. 1924.

Stems climbing sinistrorsely, not winged, unarmed, densely puberulous; leaves alternate; petioles up to 6 cm. long, puberulous; leaf blades suborbicular, about 10 cm. long and 9 cm. wide, sharply acuminate, deeply cordate at base, not lobed, not pellucid-lineolate, puberulous above, densely soft-pilosulous beneath, 13- to 17-nerved; staminate inflorescences 2–4 in an axil, unbranched, the rhachis densely pilosulous; flowers in small scorpioid racemes along rhachis, or some of them solitary, short-pedicellate, the pedicels 0.5–2 mm. long, pilosulous; perianth segments oblong, 2–2.5 mm. long, pilosulous externally; stamens 6, all fertile, borne on perianth segments, the filaments about 0.5 mm. long, slender, the anthers 0.25 mm. long, introrse, ovary densely tomentose; capsules puberulous, oblong, nearly 3 cm. long and 11 mm. wide; seeds winged all around.

Known definitely, at least in typical form, only from Panama.

CANAL ZONE: Ancón Hill, *Killip 3035* (TYPE of *D. permollis*), *12086;* Gamboa, *Pittier 4800.* COCLÉ: between Las Margaritas and El Valle, *Woodson, Allen & Seibert 1761.* PANAMÁ: La Chorrera, *Paul 508.*

The type came from Loseria, Panama (*Hayes 726*). The type of var. *Duchassaingii* was collected samewhere in Panama by Duchassaing. As pointed out by me (Carn. Inst. Publ. 461:250. 1936), Knuth erroneously referred his new species *D. permollis* to subg. Helmia, sect. Dematostemon, whereas it actually belongs in subg. Eudioscorea, sect. Macrogynodium.

9. Dioscorea macrostachya Benth. Pl. Hartweg. 73. 1839.

Dioscorea Billbergiana Kunth, Enum. Pl. 5:354. 1850.

Dioscorea anconensis Knuth, in Fedde Repert. Sp. Nov. 28:82. 1930.

Stems climbing dextrorsely (at least in Panama specimens), glabrous, not winged, unarmed; leaves alternate; petioles slender, up to 5 cm. long, glabrous; leaf blades ovate, up to 11 cm. long and 7.5 cm. wide (or probably the lower larger), long-acuminate, cordate at base, entire, glabrous, not pellucid-lineolate, 9-nerved; staminate inflorescences usually solitary in an axil, simple or much-branched, 8–30 cm. long, the rhachis glabrous; flowers sessile, borne in small, stalked fascicles or rarely solitary; perianth segments purple, ovate, about 1 mm. long, glabrous; stamens 6, all fertile, borne on base of perianth segments, nearly sessile, extrorse; rudimentary ovary present; ovary glabrous; capsules coriaceous, obovate, 2.5–3 cm. long, 1.5–2 cm. wide, truncate at apex, glabrous, the angles sharp.

Mexico to Panama.

BOCAS DEL TORO: vicinity of Chiriquí Lagoon, *von Wedel 1223, 1273, 1488, 1604, 1760, 1778.* CANAL ZONE: Ancón Hill, *Killip 12085* (TYPE of *D. anconensis*), *12056, Seibert 370;* Chagres, *Hayes 335, 336.* COCLÉ: Llano Bonito, north of Las Margaritas, *Seibert 521;* between Las Margaritas and El Valle, *Woodson, Allen & Seibert 1732.* PANAMÁ: sabanas north of Panama City, *Paul 476.*

Knuth referred his species *D. anconensis* to subg. Helmia, sect. Dematostemon, but examination of an isotype shows definitely that the plant belongs in Eudioscorea and is synonymous with *D. macrostachya* of sect. Apodostemon.

10. Dioscorea polygonoides Humb. & Bonpl. ex Willd. Sp. Pl. 4:795. 1806.

Stems slender, glabrous, unarmed, climbing sinistrorsely; leaves alternate; petioles up to 6 cm. long, glabrous; leaf blades ovate-orbicular, up to 11 cm. long and 8.5 cm. wide, abruptly acuminate, deeply cordate at base, thin-membranous, pale yellow-green, glabrous, 11-nerved; staminate inflorescences 2–4 in an axil, 10–30 cm. long, unbranched, or rarely a little branched, the rhachis glabrous, minutely scabrous; flowers sessile in few-flowered fascicles; perianth segments green, 1.3 mm. long, ovate, united in tube at base, glabrous; fertile stamens 3, inserted on perianth segments, the filaments short, the connective broad, the anther cells disjunct, extrorse; staminodia 3, nearly as long as the filaments, bifid at apex; rudimentary style large; pistillate spikes solitary or paired, unbranched, 15 cm. long or less, glabrous; capsules oval, about 1.7 cm. long and 1.2 cm. wide, glabrous; seeds winged all around.

Mexico to Colombia and Brazil. West Indies.

CANAL ZONE: Balboa, *Standley 25232, 25281, 25419, 25555, 26466, 26489, 26984, 27149, 27164, 29285, 32141;* Las Cascadas Plantation near Summit, *Standley 25676, 25771;* Las Cruces Trail, between Fort Clayton and Corozal, *Standley 29049;* road to Corozal, *Heriberto 248;* between France Field and Catival, *Standley 30363;* Gamboa, *Pittier 4802.*

11. Dioscorea Standleyi Morton in Carn. Inst. Publ. 461:252. 1936.

Stems herbaceous, glabrous, unarmed, twining dextrorsely; leaves alternate; petiole 6–7 cm. long, glabrous; leaf blades ovate, up to 12 cm. long and 10 cm. wide, acuminate, cordate at base, glabrous, entire, 9-nerved; staminate inflorescences 1 or 2 in an axil, about 15 cm. long, the rhachis glabrous; flowers borne in short cymules, the common peduncle 2–6 mm. long, the pedicels 2–4 mm. long; perianth segments green, linear-oblong, glabrous, about 2 mm. long and 0.5 mm. wide; fertile stamens 3, the filaments connate to above middle, the anthers extrorse; staminodia none; rudimentary ovary none; pistillate inflorescence solitary, unbranched, up to 11 cm. long; staminodia none; styles connate, 1 mm. long, the stigmas short; ovary glabrous; capsule about 15 mm. long and 5 mm. wide; seeds winged all around.

Costa Rica and Panama.

CHIRIQUÍ: Bajo Chorro, *Woodson & Schery 652, 664; Davidson 61.*

IRIDACEAE

Perennial herbs from rhizomes, corms, or bulbs; leaves usually basal, frequently cauline as well, mostly narrowly linear to ensiform, sheathing at the base; equitant; flowers perfect, regular or irregular, enclosed in paired conduplicate spathes; perianth petaloid, the lobes subequal or in 2 series, free or more or less fused at the base; stamens 3, opposite the outer perianth lobes; filaments free or partially connate; anthers 2-celled, dehiscing longitudinally; ovary inferior, 3-celled with axile placentae or 1-celled with parietal placentae; style slender, 3-lobed above, sometimes winged or petaloid; fruit a loculicidally dehiscent capsule.

a. Style branches opposite the stamens, furnished with petaloid crests; rootstock a short rhizome ..... 1. NEOMARICA
aa. Style branches alternate with the stamens, without crests.
  b. Rootstock a tunicated corm; inner perianth lobes connivent; style branches dilated and somewhat petaloid ..... 2. CIPURA
  bb. All perianth segments free to the ovary; style branches subulate.
    c. Rootstock a very short rhizome or virtually lacking; inflorescences simple or loosely fascicled, the flowers obviously pedicellate and the capsules exserted from the spathes ..... 3. SISYRINCHIUM
    cc. Rootstock a fairly extensive rhizome; inflorescence paniculate, the flowers virtually sessile and the capsules included within the spathes ..... 4. ORTHROSANTHUS

Moraea, Gladiolus, Tigridia, and *Tritonia* are exotic genera occasionally encountered in cultivation.

1. NEOMARICA Sprague

NEOMARICA Sprague in Kew Bull. Misc. Inf. 1928:280. 1928.

Marica Herb. in Bot. Mag. *pl. 3809.* 1840, non Schreb.
Galathea Liebm. in Ind. Sem. Hort. Haun. 26. 1855.

Rhizomatous *Iris*-like herbs; leaves ensiform, distichous, flabellate, basal; flowering scape terminated by a large foliaceous spathe, the short flowering peduncle appearing lateral, frequently nodding, bearing few to several rather handsome white or blue flowers; perianth tube obsolete, the 6 lobes in 2 very dissimilar series, the outer obovate, spreading, the inner much smaller, pandurate, ascending, more or less convolute; stamens short, erect, the filaments distinct; pistil club-shaped, 3-celled; style subulate at the base, cyathiform with 3 prominent angles in the upper half, style crests lanceolate with the transverse stigmas at their base; capsule oblong, 3-valved, containing numerous arillate seeds.

a. Plants 6–8 dm. tall, the leaves and scapes with a single very definite midrib; inflorescences 2- to 5-flowered, the outer spathes 3.0–3.5 cm. long ..... 1. N. GRACILIS
aa. Plants 12–15 dm. tall, the leaves and scapes without definite midrib; inflorescences 3- to 6-flowered, the outer spathes 7–13 cm. long ..... 2. N. CAERULEA

Fig. 21. *Neomarica gracilis*

1. NEOMARICA GRACILIS (Herb.) Sprague in Kew Bull. Misc. Inf. 1928:280. 1928.

Marica gracilis Herb. in Bot. Mag. *pl. 3713*. 1840.
Cypella gracilis (Herb.) Klatt in Mart. Fl. Bras. 3^1:521. 1871.

Plants 6–8 dm. tall; leaves narrowly ensiform, long-acuminate, gradually narrowed to a subpetiolar base, 4–7 dm. long, 1–2 cm. broad, with a single conspicuous midrib and numerous smaller parallel veins; flowering scape 3–5 dm. long, slightly winged below, conspicuously so above; terminal spathe leaf-like, 3–3.5 dm. long, 2–3 cm. broad, with a conspicuous midrib; flowering peduncle 1–3 cm. long, bearing 2–5 pretty white or blue flowers; perianth about 5 cm. in diameter, the spathes 1–3 cm. long; capsule oblong, about 2–3 cm. long.

Mexico to northern Brazil, in wet lowland forests and savannas.

BOCAS DEL TORO: Old Bank Island, *von Wedel 2163*. CANAL ZONE: Barro Colorado Island, *Kenoyer 227;* Summit, *Lindsay 308;* Balboa, *Standley 28576*. COCLÉ: El Valle, *Allen 756*. COLÓN: Tumba Vieja, *Dodge, Steyermark & Allen 16931*.

2. NEOMARICA CAERULEA (Ker) Sprague, Kew Bull. Misc. Inf. 1928:280. 1928.

Marica caerulea Ker in Bot. Reg. *pl. 713*. 1823.
Galathea speciosa Liebm. in Ind. Sem. Hort. Haun. 26. 1855.
Cypella caerulea (Ker) Seub. ex Hook. f. in Bot. Mag. *pl. 5612*. 1866.
Galathea coerulea Liebm. ex Klatt in Mart. Fl. Bras. 3^1:519. 1871, in synon.

Plants 12–15 dm. tall; leaves narrowly ensiform, 6–9 dm. long, 3–4 cm. broad, without a definite midrib; flowering scape ensiform, 7–9 dm. long, as broad as the leaves; spathes 5–8 cm. long, terminal spathe ensiform, 5–6 dm. long; flowers 3–6, on a very short peduncle; perianth about 7–10 cm. in diameter, bright blue with brown bars at the base of the lobes.

Guiana and Brazil; widely cultivated, the Panama specimen probably an escape.

BOCAS DEL TORO: Little Bocas, *von Wedel 2546*.

2. CIPURA Aubl.

CIPURA Aubl. Hist. Pl. Guian. 1:38. *pl. 13*. 1775; Standl. Fl. Pan. Canal Zone, 116. 1928.

Marica Schreb. Gen. Pl. 1:37. 1789, non Ker.
Bauxia Neck. Elem. 3:160. 1790.

Small or mediocre herbs from a tunicated corm; leaves basal, distichous, narrowly ensiform; flowering scape terminated by a leaf-like spathe, the short flowering peduncle appearing lateral, bearing 2–4 rather pretty, very evanescent light blue or white flowers; perianth tube obsolete, the outer lobes obovate-cuneate, reflexed, the inner much shorter, persistently connivent; style filiform, the branches oblong, dilated and somewhat petaloid; capsule oblong-turbinate, 3-valved.

1. CIPURA PALUDOSA Aubl. Hist. Pl. Guian. 1:38. *pl. 13.* 1775; Standl. Fl. Pan. Canal Zone, 116. 1928.

Cipura graminea HBK. Nov. Gen. & Sp. 1:320. 1816.
Cipura humilis HBK. loc. cit. 1816.
Marica paludosa (Aubl.) Willd. in Bot. Mag. *pl. 646.* 1803.

Fig. 22. *Cipura paludosa*

Corm ovoid, covered with deep brown scales; leaves linear-ensiform, 1.5–5.0 dm. long, 0.4–0.6 cm. broad; flowering scapes filiform, 1.5–4.5 dm. long; terminal spathe linear-ensiform, 12–30 cm. long; inflorescence sessile, bearing 2–4 light blue or white flowers; perianth about 3–4 cm. in diameter; flowering spathes narrowly lanceolate, 2–4 cm. long.

Widespread from Mexico and Cuba to southern Brazil, in savannas and thickets chiefly at low elevations.

CANAL ZONE: Ancón Hill, *Standley 26335.* COCLÉ: between Aguadulce and Antón, *Woodson, Allen & Seibert 1226;* between Las Margaritas and El Valle, *Woodson, Allen & Seibert 1268.* CHIRIQUÍ: Llanos del Volcán, *Seibert 348.* PANAMÁ: Arraiján, *Woodson, Allen & Seibert 1673;* La Joya, *Dodge, Hunter, Steyermark & Allen 16910;* Río Tapia, *Maxon & Harvey 6627.*

3. SISYRINCHIUM L.

SISYRINCHIUM L. Sp. Pl. 954. 1753.

Bermudiana Adans. Fam. 2:60. 1763.
Hydastylus Dryand. ex Salisb. Trans. Hort. Soc. Lond. 1:310. 1812.
Souza Vell. Fl. Flum. 273. 1825.
Olsynium Raf. New Fl. N. Amer. 1:72. 1836.
Pogadelphia Raf. Fl. Tellur. 4:29. 1836.
Paneguia Raf. loc. cit. 34. 1836.
Echthronema Herb. in Bot. Reg. Misc. N. S. 6:85. 1843.
Eriphilema Herb. loc. cit. 1843.
Glumosia Herb. loc. cit. 1843.
Androsolen Lem. in Fl. Serres 2:146. 1846.

Spathirachis Klotzsch apud Klatt, in Linnaea 31:96. 1861.
Oreolirion Bickn. apud Wooton & Standl. in Contr. U. S. Nat. Herb. 19:147. 1915.

Small or mediocre herbs with fibrous or tuberous roots; leaves basal or both basal and cauline, ensiform to linear-ensiform, equitant; flowering stem leafless or leafy, simple or branched, bearing a cluster of few to several rather small pedicellate flowers partly enclosed by 2 more or less foliaceous equitant spathes; perianth with a short tube above the ovary, the segments equal or subequal, petaloid, more or less spreading above the base; stamens 3, inserted at the base of the perianth; filaments free or more or less connate; anthers versatile; ovary globose to turbinate or oblongoid, 3-celled; style subulate; stigmas 3; fruit a loculicidally 3-valved capsule.

a. Flowering stems bearing 1 or more leaves and usually branching at least once (except in S. *chiricanum*), only slightly winged or ancipitous.
  b. Plants caespitose, forming grass-like turfs, not greatly discoloring in drying; roots slender and fibrous, the hairs extremely inconspicuous; perianth blue to pinkish lavender, usually pale yellowish at the base, about 0.7 cm. long; stamen filaments connate to the anthers; capsules globose, about 0.3 cm. in diameter; seeds about 0.1 cm. in diameter, conspicuously reticulate-foveolate ..... 1. S. MICRANTHUM
  bb. Plants not caespitose, with the habit of a miniature *Iris*, conspicuously discoloring in drying; perianth yellow veined with brown; stamen filaments connate somewhat below the middle.
    c. Stems 1–3 dm. tall; roots chiefly short and tuberous, without conspicuous persistent hairs.
      d. Stems more or less conspicuously flexuose, branching repeatedly in mature plants; fibers of past leaves somewhat persistent, but scarcely conspicuous; leaves 0.4–0.6 cm. broad, characteristically turgid and patulous, particularly upon the stem; spathe valves ovate-lanceolate; perianth 0.8–1.0 cm. long; capsules oblongoid-subglobose, scarcely longer than broad; seeds about 0.1 cm. in diameter, lustrous and conspicuously reticulate-foveolate ..... 2. S. CONVOLUTUM
      dd. Stems straight or very slightly flexuose, simple or branching infrequently; fibers of past leaves very conspicuous and matted; leaves 0.1–0.2 cm. broad, erect; spathe valves narrowly lanceolate; perianth 1.2–1.5 cm. long; capsules oblongoid, about twice longer than broad; seeds about 0.13 cm. in diameter, opaque, smooth or very inconspicuously foveolate ..... 3. S. MANDONI
    cc. Stems 4–5 dm. tall; roots elongate and only slightly fleshy, the persistent hairs very conspicuous and matted; perianth 1.6–1.8 cm. long; capsules ovoid, sharply tapered at base and tip, about 1.5 cm. long; seeds about 0.2 cm. in diameter, conspicuously reticulate-foveolate ..... 4. S. CHIRICANUM
aa. Flowering stems leafless and unbranched, very broadly ancipitous and leaf-like, not greatly discoloring but yielding a purple dye in drying; roots elongate and fibrous; perianth about 0.8 cm. long; stamen filaments free to the ovary; capsules broadly turbinate, about 1.2 cm. long; seeds about 0.15 cm. in diameter, obscurely foveolate ..... 5. S. TINCTORIUM

1. SISYRINCHIUM MICRANTHUM Cav. Diss. Bot. 6:144. *pl. 191, fig. 2.* 1788.

Sisyrinchium micranthemum Pers. Syn. 1:50. 1805, *sphalm.*
Marica micrantha (Cav.) Ker, Irid. Gen. 22. 1827.

Small caespitose herbs usually forming grass-like turfs, not greatly discoloring in drying; roots slender and fibrous; leaves linear-ensiform, 4–9 cm. long, 0.1–0.3 cm. broad, both basal and sparse upon the flowering stems; flowering stems almost

invariably branching at least once, 7–20 cm. long, slender and only slightly ancipitous; inflorescence simple, few- to several-flowered; spathe valves very unequal, foliaceous, compressed, the outer 1–3 cm. long; perianth blue to pinkish lavender, usually yellowish at the base, rather narrow, 0.7–0.8 cm. long, minutely puberulent to glabrous at the base without; stamen filaments completely connate to the anthers, about 0.1 cm. long; capsules globose, about 0.3 cm. in diameter; seeds somewhat angular, about 0.1 cm. in diameter, conspicuously reticulate-foveolate.

Southern Mexico to Bolivia, in subalpine meadows and open woods.

CHIRIQUÍ: Volcán de Chiriquí, *Woodson, Allen & Seibert 889;* Cerro Punta, *Seibert 250;* Chiquero, *Davidson 560;* upper Río Chiriquí Viejo, *White & White 4;* El Boquete, *Pittier 2968;* Bajo Chorro, *Woodson & Schery 628.*

S. *micranthum* is closely allied to the northern S. *angustifolium* ("Blue-eyed Grass") and the southern S. *chilense,* both of which have much more open perianths and glumaceous spathes of nearly equal valves, and which possibly are no more than subspecifically distinct upon the basis of their seeds.

2. SISYRINCHIUM CONVOLUTUM Nocca, Pl. Select. Hort. Ticin. sub. *t. 1.* 1800. *Sisyrinchium alatum* Hook. and S. *iridifolium* HBK. of many authors.

Plants with the habit of a miniature *Iris,* greatly discoloring in drying; roots partly slender and fibrous and partly short and tuberous; leaves narrowly ensiform, 7–25 cm. long, 0.4–0.6 cm. broad, characteristically turgid and patulous, both basal and cauline; flowering stems branching once or repeatedly, more or less flexuose, rather inconspicuously ancipitous, 1–3 dm. tall, surrounded at the base with rather inconspicuous fibers of past leaves; inflorescence simple, few-flowered; spathe valves about equal, ovate-lanceolate, the outer 2.0–2.5 cm. long; perianth yellow veined with brown, rather narrow, 0.8–1.0 cm. long, glabrous; stamen filaments 0.4–0.6 cm. long, connate to somewhat below the middle; capsules oblongoid-subglobose, about 1 cm. long; seeds about 0.1 cm. in diameter, lustrous and conspicuously reticulate-foveolate.

Southern Mexico to Peru, in highland llanos.

CHIRIQUÍ: Boquete, *Davidson 788;* Chiriquí Viejo valley, *G. White 98;* Llanos del Volcán, *Seibert 346.*

In the herbarium of the Missouri Botanical Garden are three sheets from the collection of Bernhardi, which bear his notation "*Sisyrinchium convolutum* Nocca." While these specimens scarcely have the authenticity of actual types or isotypes, yet they represent probably plants grown by Bernhardi in his well-stocked garden at Erfurt and may rather confidently be taken as illustrative of the application of the specific name during the middle part of the past century. Bernhardi corresponded very actively with other botanists of his period and exchanged both dried specimens and seeds, such acquisitions of his now forming a little-recognized treasure of botanical antiquities in the herbarium of the Missouri Botanical Garden not duplicated elsewhere in America.

Our plants check so well with "*Sisyrinchium convolutum*" of Bernhardi's

collection, and so adequately with published descriptions and icones that our disposition has considerable claim to accuracy. Whatever they may be, they certainly are not S. *alatum* Hook. (properly S. *Marchio* Vell.) nor S. *iridifolium* HBK., as a thoughtful examination of standard references will show. The latter species is not actually a member of the yellow-flowered S. *alatum* alliance, as it is treated usually, but of the *angustifolium-chilense* complex closely related to S. *micranthum*. Its flowers are not actually yellow, but blue-striped, at least with a yellowish base as in S. *micranthum*.

The confusion of S. *convolutum* with S. *alatum* apparently is due, at least in part, to the rather hasty efforts of Baker, who determined a widely distributed specimen from Guatemala (*Heyde & Lux 3533*) as the latter species, with the possible intention for his S. *alatum* var. *guatemalense* (Handb. Irid. 130. 1892), probably referable to S. *convolutum*. True S. *alatum* of South America is a very different, much larger plant with crowded, short and incurved leaves, smaller nearly globose capsules, and slender, fibrous roots.

S. *convolutum* apparently is a rather frequent species extending from Hidalgo and Jalisco, in Mexico, possibly as far south as Ecuador. We have made no effort to disentangle a full selection of synonyms for S. *convolutum*, in view of the taxonomic confusion of the genus.

3. SISYRINCHIUM MANDONI Baker in Jour. Bot. 14:269. 1876.

Fig. 23
Sisyrinchium Mandoni

Plants with the habit of a miniature *Iris*, surrounded at the base with the matted fibers of past leaves, discoloring in drying; roots partly slender and fibrous and partly short and tuberous; leaves linear-ensiform, 9–15 cm. long, 0.1–0.2 cm. broad, erect, borne both basally and sparsely upon the stem; flowering stems 1.5–2.0 dm. tall, inconspicuously ancipitous, usually simple and straight, infrequently branching and then somewhat flexuose; inflorescence simple, 2- to 6-flowered; spathe valves subequal, lanceolate, the outer 2.5–4.0 cm. long; perianth broadly ampuliform, yellow veined with brown, 1.2–1.5 cm. long, glabrous; stamen filaments 0.4–0.6 cm. long, connate somewhat below the middle; capsules oblongoid, 1.0–1.3 cm. long, about 0.4 cm. broad; seeds subglobose, about 0.13 cm. in diameter, opaque, smooth or very inconspicuously foveolate, with a very deep micropylar pit.

Mountains of Panama, Colombia, and Bolivia.

CHIRIQUÍ: Potrero Muleto to summit, Volcán de Chiriquí, *Woodson & Schery 427;* valley of the upper Río Chiriquí Viejo, *P. White 61;* Loma Larga to summit, Volcán de Chiriquí, *Woodson, Allen & Seibert 1038.*

We have only Baker's description to support assignment of these plants to S. *Mandoni*, but the agreement is striking.

It is unfortunate that Baker did not include descriptions and measurements of seeds in his descriptions of *Sisyrinchia*, for we have been very strongly impressed by their use as diagnostic criteria. The smooth seeds of our Panamanian plants, with their deep micropylar pits, are quite unlike those of any other species known to us, and should be of considerable use in the final taxonomic disposition of the plants.

4. Sisyrinchium **chiricanum** Woodson, spec. nov.

Plantae habitu *Iridem* gracilem vel *Orthrosanthum* simulantes, fibris foliorum vetustorum basi parce persistentibus, post exsiccationem discoloratae. Radices elongatae paulo incrassatae conspicue pubescentes. Folia lineari-ensiformia, radicalia 30–40 cm. longa 0.5–1.0 cm. lata, caulinia brevioria. Caules florigeri 4–5 dm. alti inconspicue alati repetite ramosi haud flexuosi. Inflorescentia simplex 1–3-flora; spathae valvae paulo inaequales ovato-lanceolatae exteriores 2.5–4.0 cm. longae. Perianthium amplissimum luteum brunneo-nervatum 1.6–1.8 cm. longum glabrum. Staminum filamenta 0.6–0.7 cm. longa sub medio connata. Capsulae ovoideae basi apiceque angustatae ca. 1.5 cm. longae 0.9 cm. crassae; semina subglobosa ca. 0.2 cm. diametralis conspicue reticulato-foveolata.

Plants with the habit of an *Iris* or an *Orthrosanthus*, surrounded at the base with persistent fibers of past leaves, greatly discolored in drying; roots elongate and somewhat fleshy, the persistent hairs very conspicuous and matted; leaves linear-ensiform, the basal 30–40 cm. long, 0.5–1.0 cm. broad, the cauline progressively shorter to the spathes; flowering stems 4–5 dm. tall, inconspicuously alate, branching repeatedly, not flexuose; inflorescence simple, 1- to 3-flowered; spathe valves slightly unequal, ovate-lanceolate, the outer 2.5–4.0 cm. long; perianth very broadly ampuliform, yellow veined with brown, 1.6–1.8 cm. long, glabrous; stamen filaments 0.6–0.7 cm. long, connate somewhat below the middle; capsules ovoid, tapered at both base and apex, about 1.5 cm. long and 0.9 cm. thick; seeds subglobose, about 0.2 cm. in diameter, conspicuously reticulate-foveolate.

Known only from the type locality.

Chiriquí: Casita Alta to Cerro Copete, *Woodson & Schery 354* (type, in Herb. Missouri Bot. Garden); same locality, *Woodson & Schery 344* (cotype, in Herb. Missouri Bot. Garden).

Amongst species known to us, S. *chiricanum* most resembles S. *arizonicum*, at least as to general habit and roots. The capsules of the latter, however, are bluntly oblongoid, and its seeds are even larger than those of S. *chiricanum*. Both species are striking in their habit of bearing one or more leaves upon the branches of the flowering stems. The stems of S. *arizonicum* are more broadly winged than those of S. *chiricanum*.

5. Sisyrinchium tinctorium HBK. Nov. Gen. & Sp. 1:324. 1816.

Marica tinctoria (HBK.) Ker, Irid. Gen. 23. 1827.
Sisyrinchium tingens Steud. Nomencl. 2:596. 1841.
Sisyrinchium rigidum Lehm. in Hamb. Gartenz. 6:415. 1850.

Iris-like herbs 1.5–4.0 dm. tall, not greatly discoloring, but yielding a purplish

dye in drying; roots slender and fibrous; leaves all basal, lance-ensiform, 7–25 cm. long, 0.3–1.0 cm. broad; flowering scapes simple, leafless, 15–30 cm. long, very broadly ancipitous and leaf-like; inflorescence simple, 2- to 4-flowered; spathe valves very unequal, the outer leaf-like, 3–6 cm. long; perianth 0.7–0.8 cm. long, yellow veined with brown, glabrous; stamen filaments 0.4 cm. long, free to the base; capsules broadly turbinate, 0.9–1.2 cm. long; seeds about 0.15 cm. in diameter, obscurely foveolate.

Mountains of southern Mexico to Peru and Bolivia.

CHIRIQUÍ: vicinity of Casita Alta, Volcán de Chiriquí, *Woodson, Allen & Seibert 850;* Finca Lerida to Peña Blanca, *Woodson & Schery 322;* "New Switzerland", valley of Río Chiriquí Viejo, *Allen 1410;* Bajo Chorro, *Woodson & Schery 644;* same locality, *Davidson 32;* valley of upper Río Chiriquí Viejo, *P. White 66.*

The first specimen cited, *Woodson, Allen & Seibert 850,* is much smaller than the others, and may represent one of the numerous segregate species. The specimen is not in fruit, however, which we would wish to have in order to assign it to another species.

Fig. 24. *Orthrosanthus chimboracensis*

4. ORTHROSANTHUS Sweet

ORTHROSANTHUS Sweet, Fl. Austral. *pl. 11.* 1827.

Eveltria Raf. Fl. Tellur. 4:30. 1836.

Rhizomatous herbs with the habit of an *Iris* or a gigantic *Sisyrinchium;* leaves chiefly basal, but more sparse and also reduced upward on the extensively branched flowering stem, distichous, equitant; inflorescence loosely paniculate, bearing several or many rather handsome, virtually sessile, blue flowers of moderate size; perianth tube very short, the lobes subequal, spreading; stamens inserted at the base of the perianth; filaments free or connate at the very base; anthers linear, erect; ovary clavate, 3-celled; style very short; stigma branches subulate; fruit a loculicidally 3-valved capsule.

1. ORTHROSANTHUS CHIMBORACENSIS (HBK.) Sweet in Gard. Chron. 2:67. 1876.

Moraea chimboracensis HBK. Nov. Gen. & Sp. 1:322. 1816.
Moraea acorifolia HBK. loc. cit. 1816.
Moraea gladioloides HBK. loc. cit. 1816.
Sisyrinchium Moritzianum Klotzsch ex Klatt in Linnaea 31:378. 1862.

Rhizomatous herbs of moderate size, 3–10 dm. tall; leaves narrowly ensiform, chiefly basal, but also reduced and rather sparse upward on the flowering stem, 5–45 cm. long, 0.5–1.2 cm. broad; flowering stem paniculately branched and rather sparsely leafy, bearing virtually sessile clusters of few to several flowers in the axils of reduced leaves; spathes 1.0–1.5 cm. long, subequal; perianth bright blue, about 1.5–2.0 cm. long; capsules oblong-clavate, 1.0–1.5 cm. long, nearly enclosed by the spathes.

Mexico through the Andes to Peru and Bolivia, in alpine meadows.

CHIRIQUÍ: Potrero Muleto, Volcán de Chiriquí, *Davidson 1020*, *Woodson & Schery 423*; Cerro Copete, *Woodson & Schery 356*.

BURMANNIACEAE

By F. P. Jonker

Annual or perennial, saprophytic or autotrophic herbs, the autotrophic species green, the saprophytic often colorless; leaves alternate, entire, simple, without stipules, mostly reduced to small scales, the non-saprophytic species often with a radical rosette of green, linear leaves; flowers hermaphrodite, usually actinomorphic, sometimes zygomorphic; stem bearing at the top 1 flower or a simple or bifid cincinnus; inflorescence sometimes pseudo-capitate; perianth corolline, limb consisting of 2 whorls of 3 lobes, one of the whorls usually smaller, seldom lacking, tube cylindrical or trigonous, sometimes 3-winged; anthers 3 or 6, (sub)sessile in the perianth throat or hanging down with short filaments; connective broad, often appendiculate; style filiform in Burmannieae, shortly cylindrical or conical in Thismieae, branching at its apex into 3 short branches each bearing a stigma, or bearing at its apex 3 sessile stigmas; ovary inferior, 1-celled with parietal placentation, or 3-celled with axile placentation; ovules numerous, anatropous, with 2 integuments; in Burmannieae perianth limb with anthers and stigmas sometimes deciduous, lower part of perianth always persistent; in other genera of this tribe whole perianth persistent; in Thismieae perianth circumscissile, only a basal, thickened ring persistent; fruit usually capsular, sometimes fleshy in Thismieae, dehiscing irregularly, or with transverse slits or at the top; seeds numerous, small, with endosperm, sometimes with loose, reticulate testa.

About 125 species, widely distributed in the tropics of both hemispheres, also in the southern United States, neighborhood of Chicago, Ill., southern Brazil and Bolivia, Mozambique, southern China, Japan, southern Australia, New Zealand and Tasmania.

a. Anthers 3, sessile or subsessile in the perianth throat, thecae dehiscing with transverse slits; flowers actinomorphic, the perianth persistent or the tube persistent on the capsule; style as long as the perianth tube.
  b. Ovary 3-celled with axile placentation, often prominently 3-winged, but not in the Panamanian species; perianth as a whole persistent on the capsule ..... 1. Burmannia
  bb. Ovary 1-celled with 3 parietal placentas.
    c. Perianth limb deciduous; seeds subglobose, ovoid or ellipsoid; capsule dehiscing irregularly at the top or with irregular transverse slits; ovary with a gland at the top of both sides of the placenta ..... 2. Gymnosiphon

cc. Perianth limb persistent; seeds linear or sublinear, seldom ellipsoid; capsule 3-valved, dehiscing between the placentas; ovary without glands on the placentas ..... 4. APTERIA

aa. Stamens 6, hanging down in the perianth tube, thecae dehiscing longitudinally; flowers zygomorphic, the perianth circumscissile, only a small basal ring persistent on the fruit; style very short, conical ..... 3. THISMIA

1. BURMANNIA L.

BURMANNIA L. Sp. Pl. 287. 1753.
Vogelia J. F. Gmel. Syst. Nat. 2:107. 1791.
Tripterella L. C. Rich. apud Michx. Fl. Bor. Amer. 1:19. 1803.

Annual or perennial, autotrophic or saprophytic herbs, the saprophytic species often colorless; stem simple or branched, beset with scale-like leaves, in the non-saprophytic species usually with a rosette of linear leaves at the base; flowers solitary or in groups at the top of the stem or in dense terminal cymose or head-like inflorescences; perianth limb usually consisting of 6 lobes, the outer 3 being much larger, inner ones often minute, tube cylindrical-trigonous; anthers 3, subsessile in the perianth throat below the inner lobes; style filiform, branching at the top into 3 short branches, each bearing a stigma; ovary trigonous, 3-celled, placentas axile; in most species ovary and perianth tube prominently 3-winged, although not in those of Panama; ovary crowned by the persistent, dried perianth, dehiscing irregularly; seeds many, oblong or ellipsoid.

1. BURMANNIA CAPITATA (J. F. Gmel.) Mart. Nov. Gen. & Sp. 1:12. 1824; Jonker, Monogr. Burm. 69. 1938.

Anonymos capitatus Walt. Fl. Carol. 69. 1788.
Vogelia capitata J. F. Gmel. Syst. Nat. 2:107. 1791.
Tripterella capitata (J. F. Gmel.) Michx. Fl. Bor. Am. 1:19. 1803.

Fig. 25
Burmannia capitata

Annual, erect, non-saprophytic herbs 3–30 cm. high; stem usually simple, rarely branched, bearing at the apex a capituliform inflorescence; at the base of the stem usually a few linear or linear-lanceolate, rosulate, subulate leaves up to 4 mm. long; stem leaves becoming smaller and more scale-like upwards, the uppermost appressed, about 2 mm. long; inflorescence consisting of a contracted, bifid cyme appearing like a capitulum, 2- to many-flowered; bracts lanceolate, acuminate, about 2 mm. long; flowers wingless, erect, subsessile, mostly white, yellowish or pinkish, up to 4 mm. long; perianth lobes erect, outer lobes triangular with involute margin, acute, about 0.5 mm. long, inner ones linear or narrowly oblanceolate, obtuse, almost as long as the outer, tube trigonous, about 1.5 mm. long; anthers subsessile in the upper perianth tube; connective broadly triangular, thick and fleshy, with short lateral arms bearing the thecae and two obtuse membranaceous crests at the upper surface; style thick-filiform, swollen at the base, branching at the top into 3 branches each bearing a curved, funnel-shaped stigma, the shaft about 1.5 mm. long; capsule obovoid, dehiscing transversely;

seeds small, ellipsoid to oblong, acuminate, brownish yellow, about 0.3 mm. long.

Widespread in America from North Carolina to Paraguay; Antilles.

COCLÉ: Aguadulce, *Pittier 4945;* Natá, *Allen 823.* PANAMÁ: Nuevo San Francisco, *Standley 30774;* Las Sabanas, *Standley 40767;* near Río Azote Caballo, *Dodge, Steyermark & Allen 16856.*

2. GYMNOSIPHON Bl.

GYMNOSIPHON Bl. Enum. Pl. Jav. 1:29. 1827.

Cymbocarpa Miers in Proc. Linn. Soc. 1:61. 1840.
Ptychomeria Benth. in Hook. Jour. Bot. 7:14. 1855.
Benitzia Karst. in Linnaea 28:420. 1856.

Annual, erect, saprophytic herbs; stem simple or branched, 3- to many-flowered, mostly bearing a terminal, double cincinnus; leaves sessile, small, ovate or ovate-lanceolate, scale-like; flowers sessile or shortly pedicellate; perianth consisting of a tubular part and a 6-lobed limb, the outer lobes ovate, much larger than the inner; stamens 3, inserted below the inner perianth lobes; anthers sessile, the connective rather broad, exappendiculate or mucronulate, the thecae bursting with a median, horizontal slit; ovary ovoid or nearly globose; placentas 3, parietal, each bearing at both sides of the top a large, globose gland; ovules many, small, the funicles shorter than the ovules; style reaching the insertion of the stamens and branching into 3 short branches, each bearing a stigma which is often appendiculate; perianth limb deciduous below the insertion of the stamens after flowering, the upper part of the style also deciduous with the stamens; perianth tube persistent on the capsule; capsule dehiscing at the top in the Asiatic species, or irregularly in longitudinal direction with 3 clefts between the placentas in the American and African species.

a. Flowers 8–12 mm. long; inflorescence usually 3- to 17-flowered; stem robust; anther-connective 2-lobed at the top ..... 1. G. SUAVEOLENS
aa. Flowers usually smaller; inflorescence 1- to 5-flowered; stem slender; anther-connective entire ..... 2. G. PANAMENSIS

1. GYMNOSIPHON SUAVEOLENS (Karst.) Urban, Symb. Ant. 3:438. 1903; Jonker, Monogr. Burm. 189. 1938.

Benitzia suaveolens Karst. in Linnaea 28:420. 1856.
Ptychomeria suaveolens (Karst.) Schltr. in Fedde Repert. Sp. Nov. 17:257. 1921.

Plants 8–30 cm. tall; stem white, robust, usually simple, bearing a terminal, bifid, 3- to 17-flowered cincinnus, sometimes single-flowered; leaves scale-like, 1–3 mm. long, ovate or lanceolate, obtuse; bracts minute; pedicels white or vinous-purple, 2–6 mm. long; flowers 8–12 mm. long; perianth white or blue, tubular part 2.0–3.5 mm. long, limb 4–5 mm. long, outer lobes ovate, obtuse, with lanceolate lateral lobes as long as or sometimes longer than the middle lobe, inner lobes clavate, sometimes rather large and equalling the middle of the outer lobes, often thick and glandular-swollen; anthers inserted in the throat of the perianth, the connective split at the top into 2 lobes bearing the thecae; style

thick-filiform, branched at the top into three branches, each bearing a stigma with 2 long, thick-filiform appendages at the top; ovary 2.0-3.5 mm. long, obovoid, bluish gray, crowned by the cylindrical persistent part of the perianth tube, about 2-4 mm. long; seeds ovoid, funicles very short.

Widespread from southern Mexico to Brazil.

CHIRIQUÍ: valley of upper Río Chiriquí Viejo, *White & White 15, 24;* vicinity of "New Switzerland", central valley of Río Chiriquí Viejo, *Allen 1402.*

2. GYMNOSIPHON PANAMENSIS Jonker, Monogr. Burm. 109. *fig. 18.* 1938.

Plants 6–15 cm. tall; stem white, filiform, usually simple, sometimes shortly branched, beset with few, acute, lanceolate, scale-like leaves about 1 mm. long; inflorescence terminal, bifid and 3- to 5-flowered or solitary, branches up to 2 cm. long; bracts lanceolate, about 1 mm. long, 1-veined, acute to slightly acuminate; flowers about 8 mm. long; perianth white, the tubular part very short, about 1–2 mm. long, the limb about 4 mm. long, the outer lobes ovate to triangular, subobtuse, margin revolute, the inner lobes minute, hardly visible; anther connective broad, rounded-rhomboid, without appendages; style thick-filiform, bearing at the top 3 sessile emarginate stigmas with 2 hair-like appendages; ovary ellipsoid, truncate, up to 2 mm. long, the placenta glands bulging; capsule ellipsoid to subglobose, about 2 mm. long, crowned by the persistent, dried perianth tube.

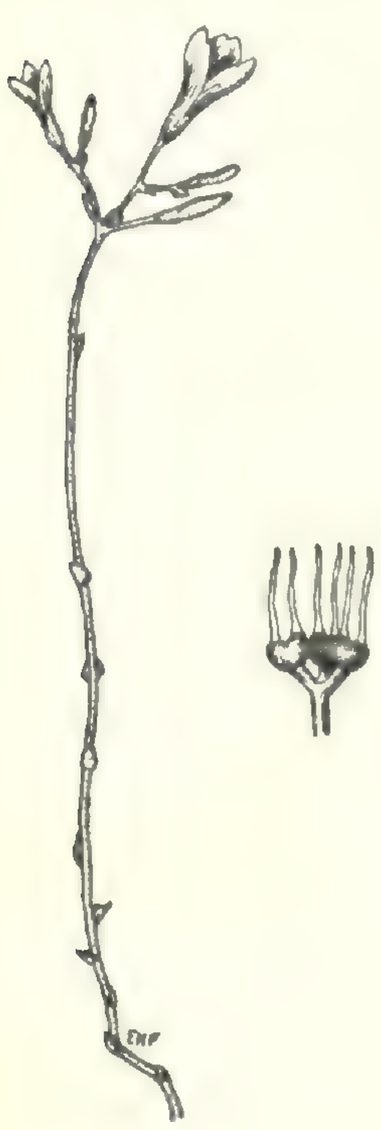

Fig. 26
Gymnosiphon panamensis

Panama, in lowland forests.

COLÓN: top of Tumba Vieja, *Dodge, Steyermark & Allen 16928.* PANAMÁ: Río La Maestra, *Allen 18.*

3. THISMIA Griff.

THISMIA Griff. in Proc. Linn. Soc. 1:221. 1844; Jonker, Monogr. Burm. 227. 1938.

Tribrachys Champ. in Thwaites, Enum. Pl. Zeyl. 325. 1864.
Rodwaya F. Muell. in Bot. Centralbl. 45:258. 1891.

Saprophytic, fleshy herbs; underground part tuberous or (not in the Panama species) coralline or vermiform; stem usually short and unbranched, sparsely beset with small scale-like leaves; flowers erect, urceolate to campanulate, subtended by one or more scale-like bracts occasionally forming an involucre; perianth lobes 6, occasionally free and of equal length, or outer lobes smaller, sometimes the inner lobes connivent or connate, with a prominent faucal annulus; anthers usually quadrangular, 6, free or connivent into a tube, hanging with short, mostly ribbon-shaped filaments at the annulus, sometimes with alternating short, triangular lobes; ovary obconical to obovoid with 3 stalked placentas in-

serted at the basal part of the ovary wall, sometimes also attached with apical stalks to the roof of the ovary; style thick and short, cylindrical or conical, persistent, bearing at its apex 3 simple or bilobate stigmas; fruit fleshy, cup-shaped, crowned by the persistent thick, fleshy, basal ring of the perianth tube and persistent style and stigmas.

1. THISMIA PANAMENSIS (Standl.) Jonker, Monogr. Burm. 234. 1938.

Ophiomeris panamensis Standl. in Jour. Wash. Acad. Sci. 7:163. 1927.

Plants 3.5–9.5 cm. tall; root system tuberous; stem erect, fleshy, leafless, 1-flowered; flower erect, without its appendages about 13 mm. long, with 4 ovate-lanceolate basal bracts up to 4 mm. long; perianth about 9 mm. long, urceolate-campanulate, very zygomorphic; inner lobes ovate, tapering at the apex to filiform appendages about 35–40 mm. long; outer lobes reflexed, ovate, rounded, without apical filiform appendages; faucal annulus prominently 3-lobed, alternating with the inner perianth lobes; anthers hanging, alternating with small triangular appendages; filaments broad, connate at their insertion with the triangular appendages into a short tube; thecae oblong, parallel; connective sagittate above the thecae, bilobed below the anthers; ovary about 2 mm. long, obovate; placentas parietal, inserted on very short stalks; style thick-filiform, conical at the base, papillose in its lower part, hairy at the apex, and there divided into 3 linear stigmas; fruit fleshy, obconical, about 3 mm. long, crowned by the persistent perianth tube and style; seeds numerous, shorter than the funicles.

Panama, in dense lowland forest.

CANAL ZONE: Barro Colorado Island, *Zetek s. n.*, *Kenoyer 247;* along Pearson Trail, *Dodge 3484;* along Shannon Trail, *Dodge 3460*, *Woodson & Schery 988.*

4. APTERIA Nutt.

APTERIA Nutt. in Jour. Acad. Phila. 7^1:64. *t. 9.* 1834.

Nemitis Raf. Fl. Tellur. 4:33. 1836.
Stemoptera Miers in Proc. Linn. Soc. 1:62. 1840.

Small erect saprophytic annual herbs; roots short and thin; stem simple or branched, 1- or sparsely-flowered; leaves small, sessile, ovate or lanceolate, scale-like; flowers rather large, often inclined or nodding; perianth campanulate or hypocraterimorphous, lobes 6, the outer ovate, the inner narrower than the outer but of the same length, linear-lanceolate, tubular part more than 3 times the length of the lobes; stamens inserted in sacks in the perianth tube below the inner perianth lobes; filaments short, thick, the base inserted in the sacks of the perianth, bearing at the external side a large, 2-lobed wing, the lobes rounded at the apex; filaments at the top forked into the broad connective; thecae bursting transversely; ovary ovoid, slightly narrowed at the top into the filiform style, style reaching the insertion of the stamens and there dividing into three short branches, each bearing a dish-shaped stigma, often beset with germinating pollen-

grains; capsule 3-valved, dehiscing between the placentas, crowned by the wholly persistent, rolled perianth; seeds numerous, minute, oblong to ovoid or ellipsoid, sometimes slightly curved, with a loose reticulate testa.

1. APTERIA APHYLLA (Nutt.) Barnh. ex Small, Fl. Southeast. U. S. ed. 1, 309. 1903; Jonker, Monogr. Burmann. 205. 1938.

Lobelia aphylla Nutt. in Jour. Acad. Nat. Sci. Phila. 5:297. 1822.
Apteria setacea Nutt. loc. cit. 7[1]:64. 1834.
Apteria boliviana Rusby in Bull. N. Y. Bot. Gard. 4:447. 1907.

Plants 5–25 cm. tall; stems simple or sometimes branched, glabrous, terete, the upper part purplish, the underground part white; leaves lanceolate to ovate-lanceolate, acuminate, sessile, purplish, scale-like, about 1.5–3 mm. long and 1 mm. broad; stem 1- or sparsely-flowered, sometimes with few loose-flowered cymes at the top; flowers nodding or horizontal, 8–13 mm. long; perianth campanulate, blue, violet, or purplish, sometimes white, darker in the ovary and at the tips of the lobes, often fading to white toward the base but with darker longitudinal stripes (honey-guides), outer lobes ovate, inner lobes lanceolate to linear-lanceolate, as long as the outer lobes, obtuse, tubular part 3 times the length of the lobes; stamens inserted in crescent-shaped sacks; connective-arms broader than the filaments, wings broader than the stamens; stigmas patelliform, margin papillate, often stuck with clusters of pollen tubes; ovary obovoid, reaching a fifth of the length of the whole flowers; capsule ovoid or obovoid, sometimes nearly globose, 4–6 mm. long, crowned by the dried perianth; seeds brown, reticulate, angulate or acute at both sides, often slightly curved.

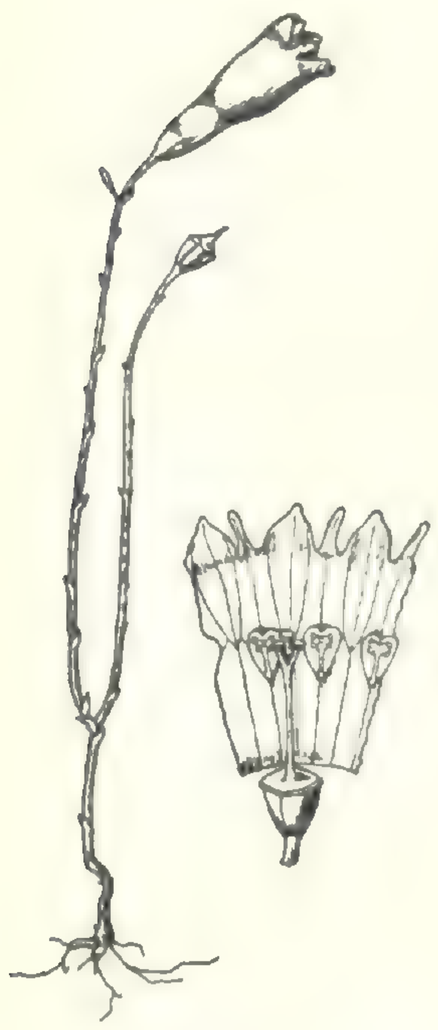

Fig. 27
Apteria aphylla

Gulf coast of United States, Mexico to Brazil and Bolivia; Cuba, Haiti, Puerto Rico.

BOCAS DEL TORO: Old Bank Island, *von Wedel 2111*.

MUSACEAE

Herbs, frequently of gigantic size, with rhizomes and fibrous or tuberous roots, the stems often very short or formed by the closely imbricate bases of the sheathing petioles; leaves spiral or distichous, with sheathing petioles, without ligules; inflorescence with large foliaceous or somewhat petalaceous bracts subtending cincinnal clusters of few to numerous flowers; flowers zygomorphic, perfect or unisexual; perianth of 2 separate series of 3 more or less united segments each, both petalaceous or the outer somewhat sepalaceous; stamens 5, rarely 6, free, the outermost occasionally staminodial; anthers 2-celled, narrowly linear, longitudinally dehiscent; ovary inferior, 3-celled, each bearing 1 to many ovules; style filiform; fruit fleshy and indehiscent or capsular and loculicidally 3-valved; seeds occasionally arillate.

Heliconia, the only native genus of this family in Panama, is common in forests at all but the highest elevations, and is known popularly as *Platanillo,* or "Wild Banana" and "Wild Plantain" by the West Indians. One of the chief industries of the Republic is the cultivation and exportation of the Banana, *Musa sapientum* L. *M. paradisiaca* L., the Plantain, or *Plátano,* is an omnipresent fruit for cooking. *M. textilis* Née, the Manila Hemp or *Abacá,* probably is destined to become an important economic crop plant. *M. Cavendishii* Lamb. and *M. Ensete* Gmel. are encountered in cultivation. *Ravenala madagascarensis* Sonn., the Traveler's Tree, or *Arbol de Viájero,* is a curious, flat-sided ornamental occasionally planted for striking landscape effects.

The taxonomy of the Musaceae is in a very unsatisfactory state at present. Particularly is this true of *Heliconia,* the species of which have been split so finely and juggled between *Heliconia* and the illegitimate *Bihai* with such ambidexterity that a final solution will come only with the most painstaking and intimate study. In the absence of such an extensive treatment, the account which follows must be regarded as provisional.

1. HELICONIA L.

HELICONIA L. Mant. 2:147. 1771.

Bihai Mill. ex Adans. Fam. 2:67. 1763; O. Ktze. Rev. Gen. 2:684. 1891; Griggs in Bull. Torrey Club 42:315-330. 1915, *et alibi.*
Bihaia O. Ktze. Rev. Gen. 2:684. 1891, *nom. altern.*
Heliconiopsis Miq. Fl. Ind. Bat. 3:590. 1858.

Rhizomatous herbs with the habit of a Canna or a Banana, mediocre or very large; leaves distichous, frequently very large; inflorescence spiciform, with conspicuous distichous, more or less conduplicate bracts subtending the clusters of flowers, erect or pendulous; flowers perfect; perianth segments in 2 series, the inner petals more or less connate, sometimes very unequal, the outer sepals separate

and more or less adnate to the corolla; stamens 5 or 6, the sixth sometimes staminodial and petaloid; ovary inferior, 3-celled; fruit a loculicidally dehiscent capsule, somewhat fleshy and berry-like.

a. Bracts closely imbricated for virtually their entire length at anthesis, concealing the rachis, except occasionally at the very base; plants very massive, 3–6 m. tall, resembling a Banana.
  b. Inflorescence erect or only slightly deflexed, somewhat cylindric in fruit; bracts ovate, broadest at the base, somewhat ascending or horizontally spreading, usually somewhat greenish at the keel; plants 3–4 m. tall ..... 1. H. IMBRICATA
  bb. Inflorescence pendulous, greatly compressed in fruit; bracts rhomboid, broadest distinctly above the base, somewhat deflexed at anthesis, deep red throughout; plants 4–6 m. tall ..... 2. H. MARIAE
aa. Bracts imbricated only at their gradually decurrent bases at anthesis, thus concealing the true rachis except occasionally at the very base, ovate to ovate-lanceolate, acuminate, uniformly ascending in both flower and fruit, usually reddish with greenish or yellowish margins and keel, occasionally wholly reddish or yellowish; inflorescence erect; plants stout, 2.5–6.0 m. tall, resembling a Banana ..... 3. H. BIHAI
aaa. Bracts relatively distant, only rarely somewhat imbricated at anthesis, the bases not decurrent nor concealing the rachis.
  b. Bracts coriaceous, broadened at the base, thus concealing the flowering and fruiting pedicels; plants relatively stout, 1.5–5.0 m. tall, resembling a Banana.
    c. Inflorescences erect; bracts spiral*, the lowermost more or less expanded and leaf-like.
      d. Inflorescence glabrous or inconspicuously puberulent.
        e. Inflorescence shortly pedunculate, the lowermost bracts usually bearing conspicuous leafy blades; perianth about 3.5 cm. long ..... 4. H. LATISPATHA
        ee. Inflorescence long-pedunculate, the lowermost bracts usually only slightly expanded and leafy; perianth about 4.5 cm. long ..... 5. H. LANKESTERI
      dd. Inflorescence densely ferruginous-tomentose to glabrate, the lowermost bract usually bearing a conspicuous leafy blade; perianth about 5.5 cm. long ..... 6. H. VILLOSA
    cc. Inflorescences pendent.
      d. Bracts horizontally spreading or somewhat ascending at anthesis, the lowermost more or less expanded and leaf-like; inflorescence inconspicuously puberulent to glabrate ..... 7. H. PLATYSTACHYS
      dd. Bracts conspicuously deflexed at anthesis, apparently never foliaceous.
        e. Inflorescence rather inconspicuously puberulent; bracts very broadly ovate, nearly as broad as long, obtuse to broadly acute ..... 8. H. ROSTRATA
        ee. Inflorescence very conspicuously ferruginous-pubescent, the lower rachis strikingly hirsute; bracts ovate-lanceolate, less than half as broad as long, acuminate to narrowly acute ..... 9. H. VELLERIGERA
  bb. Bracts membranaceous in flower, somewhat coriaceous in fruit, somewhat narrowed at the base, thus revealing the flowering and fruiting pedicels; plants relatively slender, 1–3 m. tall, resembling a Canna.
    c. Bracts laxly spreading in flower, nearly horizontal or somewhat deflexed in fruit, the lowermost occasionally more or less expanded and foliaceous; berries broader than long; leaves relatively broad, abruptly acuminate ..... 10. H. SUBULATA
    cc. Bracts sharply ascending in flower, laxly spreading in fruit; berries about as long as broad; leaves relatively narrow, gradually acuminate ..... 11. H. PSITTACORUM

*Difficult to observe in pressed specimens. The spiral appearance seems to be due to a secondary twisting of the rachis, the taxy of the bracts actually being distichous.

Fig. 28
Heliconia imbricata

1. HELICONIA IMBRICATA (O. Ktze.) Baker in Ann. Bot. 7:191. 1893.

Bihai imbricata O. Ktze. Rev. Gen. 2:684. 1891.
Bihai reticulata Griggs in Bull. Torrey Club 31:446. 1904.
Bihai densa Griggs, loc. cit. 42:320. 1915.
Heliconia reticulata (Griggs) Winkler in Engl. & Prantl, Nat. Pflanzenfam. 15a:536. 1930.
Heliconia densa (Griggs) L. B. Smith in Contr. Gray Herb. 124:6. 1939.

Plants stout, resembling a Banana; leaves broadly oblong, 75–100 cm. long, 20–40 cm. broad, acute at the tip, rounded or somewhat cordate at the base, green, the petiole 60–70 cm. long; inflorescence erect or essentially so, the stout peduncle 3–20 cm. long, bracts usually 15–20, ovate, broadest at the base, acute, 4–5 cm. long, 5–6 cm. broad, not greatly compressed, sparsely pilosulose, somewhat ascending or horizontally spreading, dark red, usually somewhat greenish at the keel, closely imbricated virtually their entire length and concealing the rachis; perianth 2.0–2.5 cm. long; fruits subglobose, about 1 cm. thick.

Costa Rica and Panama, in lowland forest.

BOCAS DEL TORO: Isla Colón, *von Wedel 400.* CHIRIQUÍ: Puerto Armuelles, *Woodson & Schery 862.* COLÓN: Dos Bocas, *Pittier;* Porto Bello, *Shannon;* upper Río Pequení, *Fairchild & Jobbins 2460.*

2. HELICONIA MARIAE Hook. f. in Jour. Linn. Soc. Bot. 7:69. 1864.

Heliconia elegans Peters. in Mart. Fl. Bras. 3^3:12. 1890.
Heliconia Wagneriana Peters. loc. cit. 13. 1890.
Heliconia conferta Peters. loc. cit. 1890.
Bihaia Mariae (Hook. f.) O. Ktze. Rev. Gen. 2:684. 1891.
Bihaia Wagneriana (Peters.) O. Ktze. loc. cit. 685. 1891.
Bihaia conferta (Peters.) O. Ktze. loc. cit. 1891.
Bihaia elegans (Peters.) O. Ktze. loc. cit. 1891.
Bihai punicea Griggs in Bull. Torrey Club 42:321. 1915.
Heliconia punicea (Griggs) L. B. Smith in Contr. Gray Herb. 124:6. 1939.

Extremely robust plants resembling Banana or Manila Hemp; leaves broadly oblong, 100–120 cm. long, 20–35 cm. broad, rounded at the base, the petioles of about equal length; inflorescence pendulous, the peduncle stout, 10–20 cm. long, glabrous or pilosulose; bracts ovate-rhombic, broadest distinctly above the base, acute to obtuse, 4–12 cm. long, 4–6 cm. broad, deep red, pilosulose to glabrate, closely imbricated virtually their entire length and concealing the rachis except at the very base; perianth 2–3 cm. long, red; fruits subglobose, deep purple, about 0.5 cm. thick.

Nicaragua to Colombia, in lowland forests and thickets.

CANAL ZONE: between Gorgona and Gatún, *Pittier 2290;* Cerro Gordo, *Standley 26000;* Empire to Mandinga, *Piper 5437;* Matachín, *Kuntze 1920.* DARIÉN: Boca de Cupe, *Allen 879.* PANAMÁ: Tabernilla, *Cowell 272.*

This is the most magnificent of described Heliconias. Because of the aspect of the broad, flat, deep red inflorescences, it has been called "Beef-steak Heliconia."

3. HELICONIA BIHAI L. Mant. 2:211. 1771.

Musa Bihai L. Sp. Pl. 1043. 1753.
Heliconia caribaea Lam. Encycl. Meth. Bot. 1:426. 1785.
Heliconia indica Lam. loc. cit. 1785.
Heliconia Biahij Vell. Fl. Flum. 3: *pl. 19*. 1827.
Heliconia buccinata Roxb. Fl. Ind. 1:670. 1832.
Heliconiopsis amboinensis Miq. Fl. Ind. Bat. 3:590. 1858.
Heliconia austro-caledonica Vieill. in Ann. Sci. Nat. Bot. IV. 16:47. 1861.
Heliconia Seemannii van Houtte, Cat. 76, 183. 1875.
Heliconia aureo-striata Bull. Cat. 18. 1881.
Heliconia Bourgaeana Peters. in Mart. Fl. Bras. 3^3:14. 1890.
Heliconia Poeppigiana Eichl. ex Peters. loc. cit. 18. 1890.
Bihaia Bourgeauana (Peters.) O. Ktze. Rev. Gen. 2:685. 1891.
Heliconia Borinquena Griggs in Bull. Torrey Club 30:658. 1903.
Heliconia Champneiana Griggs, loc. cit. 657. 1903.
Heliconia elongata Griggs, loc. cit. 653. 1903.
Heliconia rutila Griggs, loc. cit. 657. 1903.
Heliconia purpurea Griggs, loc. cit. 656. 1903.
Bihai Bihai (L.) Griggs, loc. cit. 31:445. 1904.
Bihai Borinquena Griggs. loc. cit. 1904.
Bihai Champneiana Griggs, loc. cit. 1904.
Bihai elongata Griggs, loc. cit. 1904.
Bihai purpurea Griggs, loc. cit. 1904.
Bihai rutila Griggs, loc. cit. 1904.
Heliconia barqueta Loesn. in Verh. Bot. Ver. Brandenburg 51:18. 1909.
Bihai barqueta (Loesn.) Griggs in Bull. Torrey Club 42:324. 1915.

Stout plants attaining 6 m.; leaves broadly oblong, 90–120 cm. long, 20–30 cm. broad, broadly obtuse at the base; inflorescences erect, the peduncle stout, 15–50 cm. long; bracts ovate to ovate-lanceolate, long-acuminate, obtusely perfoliate at the base, thus concealing the true rachis, the tips uniformly ascending in both flower and fruit, usually reddish with greenish or yellowish margins and keel, occasionally wholly reddish or yellowish, glabrous or essentially so; perianth 4–6 cm. long, greenish; fruit oblongoid, about 1.5 cm. long.

Mexico to Peru and Brazil; Antilles; also introduced and escaped in Oceania. In lowland forests and thickets.

CANAL ZONE: Las Cascadas, *Dodge & Hunter 8658;* Gatún, *Hayes 352;* Mandinga, *Piper 5438;* between Gorgona and Gatún, *Pittier 2291;* between France Field and Cativa1, *Standley 30347.* CHIRIQUÍ: San Bartolomé, *Woodson & Schery 892.* COCLÉ: Penonomé, *Williams 631;* La Pintada, *Hunter & Allen 455a;* La Mesa, *Allen 2708.*

Extremely abundant. This is the showiest of Panamanian Heliconias, and the colorful spikes frequently are cut for house decoration.

Fig. 29. *Heliconia Bihai*

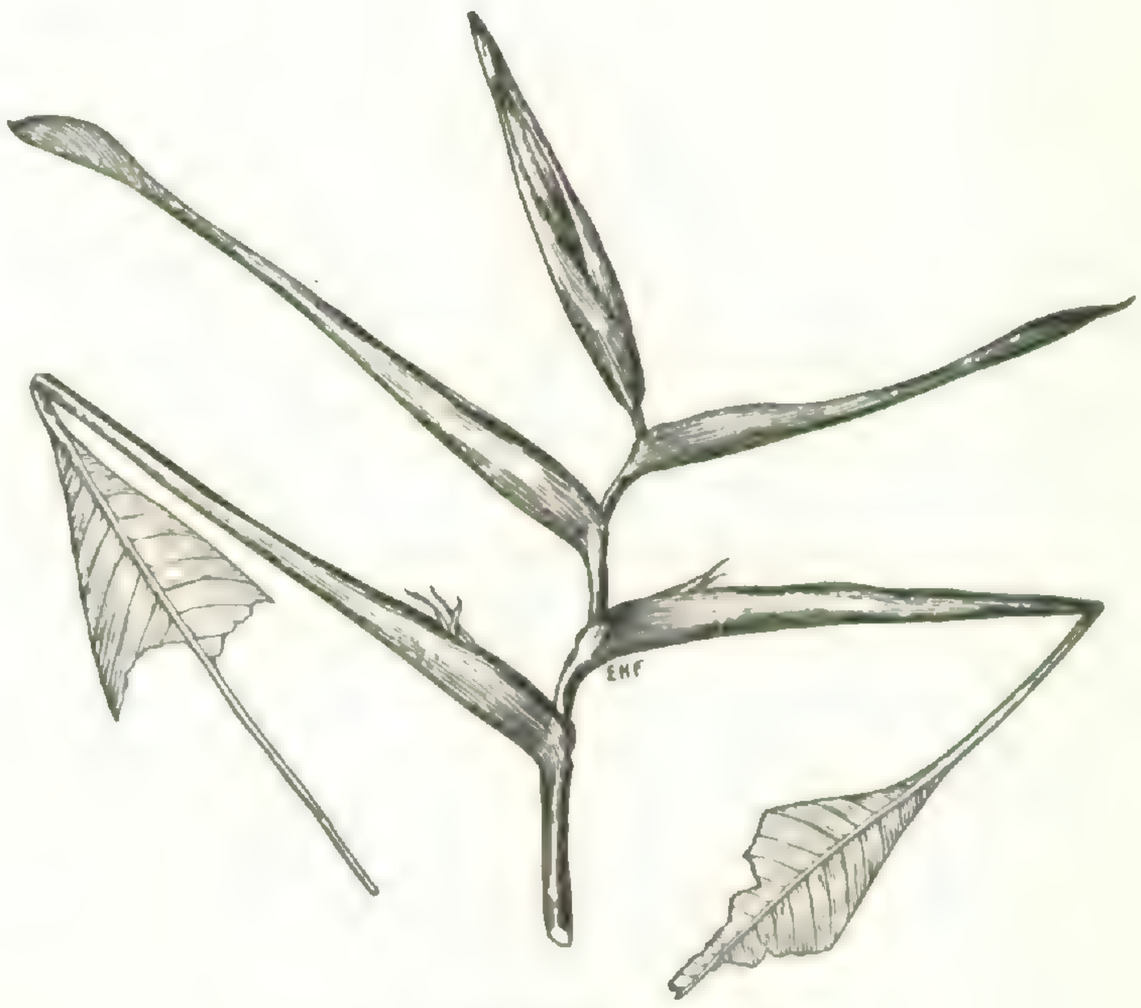

Fig. 30. *Heliconia latispatha*

4. HELICONIA LATISPATHA Benth. Bot. Voy. Sulphur, 170. 1844.

Heliconia meridensis Klotzsch in Linnaea 20:462. 1847.
Bihai latispatha (Benth.) Griggs in Bull. Torrey Club 31:445. 1904.

Stout plants 1.5–3.0 m. tall; leaves broadly oblong, 60–90 cm. long, 13–18 cm. broad, obtuse at the base, the sheath of about equal length; inflorescence erect or nearly nodding, the peduncle rather slender, 15–30 cm. long, glabrous or very inconspicuously pilosulose; bracts lanceolate, long-acuminate, broadest toward the base, the lowermost produced into more or less conspicuous leafy blades, the upper 12–18 cm. long, 2–3 cm. broad, yellow or golden-orange more or less suffused with red, glabrous; perianth about 4 cm. long, greenish yellow.

Mexico to Colombia and Venezuela, in lowland thickets and open forest.

CANAL ZONE: Summit, *Standley 29997*; Culebra, *Pittier 4062*; Ft. Sherman, *Standley 31055*; Gamboa, *Standley 28326*. COLÓN: Dos Bocas, *Pittier 4207*. PANAMÁ: Las Sabanas, *Paul 219*; Río Tecúmen, *Standley 26665*; Río Tapia, *Standley 28266*; Pedro González, Islas Perlas, *Allen 2603*; Vacamonte Pt., *Allen 2960*.

One of the most common species of the Isthmus. Widely known as *Platanillo*.

5. HELICONIA LANKESTERI Standl. in Jour. Wash. Acad. Sci. 17:162. 1927.

Stout herbs 1.5–2.5 m. tall, essentially glabrous throughout; leaves oblong-elliptic, 50–75 cm. long, 20–25 cm. broad, abruptly and shortly acuminate, rounded at the base; inflorescences erect, long-pedunculate (20–25 cm. long),

deltoid, 20–45 cm. long and broad, the rachis more or less flexuose at maturity, glabrous or with a few inconspicuous ferruginous trichomes, the internodes rather short; bracts almost overlapping at anthesis, narrowly oblong-lanceolate, broadly acute to obtuse, the lowermost somewhat expanded and foliaceous, 10–32 cm. long, ascending at anthesis, red or yellow; perianth about 4.5 cm. long, red or deep yellow, glabrous; fruits subglobose, about 1 cm. in diameter.

Highland forests of Costa Rica and western Panama.

CHIRIQUÍ: upper valley of Río Chiriquí Viejo, *Allen 1594;* Bajo Chorro, *Davidson 62.*

6. HELICONIA VILLOSA Klotzsch in Linnaea 20:463. 1847.

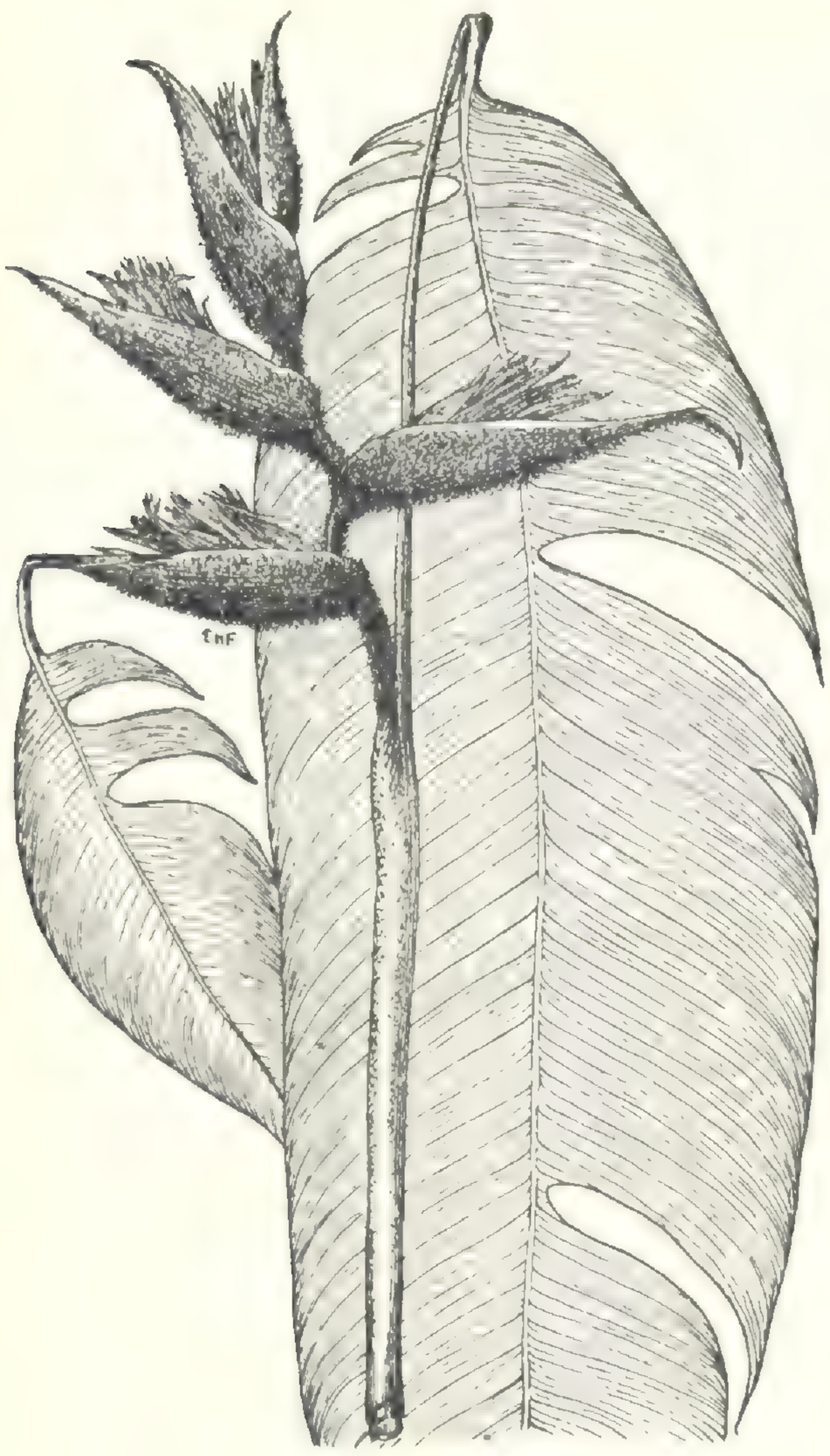

Fig. 31. *Heliconia villosa*

Heliconia dasyantha K. Koch & Bouché, Ind. Sem. Hort. Berol. App. 12. 1854.
Bihaia villosa (Klotzsch) O. Ktze. Rev. Gen. 2:685. 1891.
Bihaia dasyantha (K. Koch & Bouché) O. Ktze. loc. cit. 1891.
Heliconia tortuosa Griggs in Bull. Torrey Club 30:650. 1903.
Bihai tortuosa Griggs, loc. cit. 31:445. 1904.
Heliconia nutans Woodson in Ann. Missouri Bot. Gard. 26:276. 1939.

Stout herbs 1.5–3.0 m. tall; leaves broadly oblong, 30–100 cm. long, 10–30 cm. broad, obtuse or rounded at the base, frequently with a deep bronze cast beneath; inflorescences erect or nodding in fruit, the peduncle slender, 15–30 cm. long, more or less ferruginous-tomentose to glabrate, the rachis tomentose to glabrate, more or less flexuose; bracts rather distant, oblong-lanceolate, broadest near the base, acuminate, the lowermost expanded into a more or less conspicuous leafy blade, the upper 7–15 cm. long, 3–6 cm. broad, deep red or orange, ferruginous-tomentose to glabrate; perianth 5.0–5.5 cm. long, greenish-yellow; fruits broadly trigonal, about 1 cm. long, deep purple.

Honduras to Brazil, in highland forests.

CANAL ZONE: Río Boquerón, *Hunter & Allen 659;* Barro Colorado Island, *Kenoyer 230;* Caño Quebrado, *Pittier 6827.* CHIRIQUÍ: Volcán de Chiriquí, *Woodson, Allen & Seibert 968;* El Boquete, *Pittier 2936.* COCLÉ: El Valle, *Allen 1818.*

7. HELICONIA PLATYSTACHYS Baker in Ann. Bot. 7:194. 1893.

Bihai platystachys (Baker) Griggs in Bull. Torrey Club 31:445. 1904.
Bihai marginata Griggs, loc. cit. 42:323. 1915.
Heliconia marginata (Griggs) Pittier, Man. Pl. Usual. Venez. 299. 1926.

Stout plants 2–5 m. tall; leaves broadly oblong, rounded at the base, 60–130 cm. long, 15–30 cm. broad; inflorescences pendulous, the peduncle relatively stout, 15–35 cm. long, glabrous or essentially so, the rachis strongly flexuous; bracts ovate-lanceolate, long-acuminate, 8–30 cm. long, 2–5 cm. broad, deep yellow, essentially horizontal; perianth 3–4 cm. long, greenish-yellow; fruits broadly oblongoid, about 1 cm. long.

Guatemala to Colombia and Venezuela, in lowland forest and thickets.

DARIÉN: Boca de Cupe, *Allen 914*. CHIRIQUÍ: San Bartolomé, *Woodson & Schery 887, 891*; Puerto Armuelles, *Woodson & Schery 864*. PANAMÁ: Vacamonte Pt., *Allen 2959*.

8. HELICONIA ROSTRATA R. & P. Fl. Peruv. 3:71. *pl. 305*. 1803.

Heliconia pendula Wawra in Oesterr. Bot. Zeitschr. 13:8. 1863.
Heliconia curtispatha Peters. in Mart. Fl. Bras. 3^3:15. 1890.
Bihaia pendula (Wawra) O. Ktze. Rev. Gen. 2:685. 1891.
Bihaia curtispatha (Peters.) O. Ktze. loc. cit. 1891.
Bihai rostrata (R. & P.) Griggs in Bull. Torrey Club 31:445. 1904.
Bihai longa Griggs, loc. cit. 446. 1904.
Heliconia longa (Griggs) Winkler in Engl. & Prantl, Nat. Pflanzenfam. 15a:536. 1930.

Stout plants 3–5 m. tall; leaves broadly oblong, 45–150 cm. long, 15–40 cm. broad, broadly obtuse at the base; inflorescences pendulous, the peduncle stout, 30–90 cm. long, rather inconspicuously puberulent, the rachis flexuous; bracts very broadly ovate, obtuse to broadly acute at the tip, broadest and somewhat cordate slightly below the middle, obtusely narrowed to the base, 4–9 cm. long, 4–8 cm. broad, minutely puberulent, deep crimson, strongly reflexed at anthesis; perianth 3–4 cm. long, greenish-yellow; fruits broadly trigonal, 1 cm. long, purplish-blue.

Nicaragua to Peru, in lowland forest and thickets.

CANAL ZONE: Gatún, *Standley 27234*; between France Field and Catival, *Standley 30425*; Ft. Sherman, *Standley 31117*; Frijoles, *Piper 6036*. DARIÉN: Marraganti, *Williams 686*.

9. HELICONIA VELLERIGERA Poeppig, Reise Chile 2:295. 1836.

Bihaia vellerigera (Poeppig) O. Ktze. Rev. Gen. 2:685. 1891.

Stout herbs attaining 3 m.; leaves broadly oblong, somewhat cordate at the base, 75–150 cm. long, 20–50 cm. broad, usually bronze-tinged beneath; inflorescences pendulous, the peduncle stout, 15–20 cm. long, very densely clothed with long ferruginous hairs, the rachis strongly flexuous; bracts ovate-lanceolate, obtuse or somewhat cordate at the base, gradually acuminate, densely ferruginous-villous to glabrate, deep crimson, 6–15 cm. long, 3–5 cm. broad; perianth about 3 cm.

Fig. 32. *Heliconia vellerigera*

long, densely clothed with rather long golden hairs; fruits oblongoid, 1 cm. long, deep blue-purple.

Costa Rica to Peru, in highland forest.

COCLÉ: El Valle de Antón, *Woodson & Schery 205, Allen 1818;* Las Minas, *Allen 2707.* PANAMÁ: Río Boquerón, *Hunter & Allen 659;* Cerro Campana, *Allen 2425.*

Probably the most striking of all species of *Heliconia* because of the dense, golden indument of the flowers.

10. HELICONIA SUBULATA R. & P. Fl. Peruv. 3:70. *pl. 303b.* 1802.

Heliconia angusta Vell. Fl. Flum. 3:*pl. 20.* 1827.
Heliconia acuminata L. C. Rich. in Nova Acta Acad. Nat. Cur. 15:Suppl. *t. 11-12.* 1831.
Heliconia Choconiana S. Wats. in Proc. Amer. Acad. 23:284. 1888.
Bihaia acuminata (L. C. Rich.) O. Ktze. Rev. Gen. 2:684. 1891.
Bihai choconiana (S. Wats.) Griggs in Bull. Torrey Club 31:445. 1904.

Plants relatively slender, resembling a Canna, 1–3 m. tall; leaves elliptic-oblong, obtuse or broadly acute at the base, 30–100 cm. long, 12–20 cm. broad; inflorescence erect or slightly nodding in fruit, the peduncle relatively slender, 6–45 cm. long, glabrous, the rachis straight or slightly flexuose; bracts narrowly lanceolate, acuminate, 4–15 cm. long, 1–2 cm. broad, laxly spreading in flower, nearly horizontal or somewhat deflexed in fruit, glabrous or essentially so, green to yellowish or reddish; perianth 3–4 cm. long, greatly exserted, greenish, yellowish, or reddish; fruit broadly trigonal, conspicuously truncate, 0.4–0.5 cm. long, 0.5–0.7 cm. broad, deep purple.

Guatemala to Brazil and Bolivia; Antilles. In forests and thickets chiefly at lower elevations.

BOCAS DEL TORO: Changuinola Valley, *Dunlap 138a;* Talamanca Valley, *Carleton 138;* Río Cricamola, *Woodson, Allen & Seibert 1431;* Chiriquí Lagoon, *von Wedel 1023.* CANAL ZONE: Chagres, *Fendler 443;* Barro Colorado Island, *Standley 31291;* Gatún, *Hayes 90;* Ft. Randolph, *Standley 28669;* Quebrada Ancha, *Dodge, Steyermark & Allen 16993.* CHIRIQUÍ: San Bartolomé, *Woodson & Schery 889.* COCLÉ: El Valle de Antón, *Allen 1669.* COLÓN: Catival, *Standley 30181;* Colón, *Kuntze 1844.* DARIÉN: between Pinogana and Yaviza, *Allen 259;* Garagará, *Pittier 5683;* Cana-Cuasi Trail, *Terry & Terry 1431.* PANAMÁ: between Panamá and Chepo, *Hunter, Dodge, Steyermark & Allen 16654;* Cerro Campana, *Allen 2216.* SAN BLAS: Puerto Obaldía, *Pittier 4404.*

Fig. 33. *Heliconia subulata*

Fig. 34. *Heliconia psittacorum*

11. HELICONIA PSITTACORUM L. f. Suppl. 158. 1781.

Heliconia hirsuta L. f. loc. cit. 158. 1781.
Heliconia marantifolia G. Shaw, Cimel. Phys. 74. *t. 38.* 1796.
Heliconia cannoidea L. C. Rich. in Nova Acta Nat. Cur. 15: Suppl. *t. 9–10.* 1831.
Heliconia aurantiaca Ghiesbr. ex Lem. in Illustr. Hortic. *pl. 332.* 1862.
Bihaia psittacorum (L. f.) O. Ktze. Rev. Gen. 2:684. 1891.
Bihaia hirsuta (L. f.) O. Ktze. loc. cit. 1891.
Bihaia cannoidea (L. C. Rich.) O. Ktze. loc. cit. 685. 1891.
Heliconia hirsuta var. *cannoidea* (L. C. Rich.) Baker in Ann. Bot. 7:197. 1893.
Bihai aurantiaca (Ghiesbr.) Griggs in Bull. Torrey Club 31:445. 1904.
Bihai straminea Griggs, loc. cit. 42:327. 1915.
Heliconia straminea (Griggs) Standl. in Jour. Wash. Acad. Sci. 17:162. 1927.

Slender herbs with the habit of a Canna, 1–3 m. tall; leaves oblong-lanceolate, rounded at the base, rather gradually acuminate, 15–30 cm. long, 6–12 cm. broad; inflorescence erect, the peduncle slender, 3–15 cm. long, minutely puberulent to glabrate, the rachis slightly flexuous; bracts narrowly lanceolate, long-acuminate, 3–12 cm. long, 1.0–1.5 cm. broad, sharply ascending in flower, laxly spreading in fruit, membranaceous, minutely puberulent to glabrate, green to yellow or red, frequently variegated; perianth about 2 cm. long, yellow or white tipped with green, minutely puberulent to glabrate; fruits broadly oblongoid, about 1 cm. long and broad, deep blue-purple.

Mexico to Brazil and Peru; Antilles. In lowland forest and thickets, occasionally at higher elevations.

BOCAS DEL TORO: Fish Creek, *von Wedel 2389.* CANAL ZONE: Cañon of Río Chagres, *Dodge & Allen 17343;* between Corozal and Ancón, *Pittier 6725;* Gamboa, *Wheeler & Zetek s. n.;* Balboa, *Standley 25435;* Gatún, *Hayes 90;* Miraflores Lake, *P. White 240.* COCLÉ: La Mesa, *Allen 2692.* DARIÉN: Chepigana, *Terry & Terry 1423.* PANAMÁ: Río Tecúmen, *Hunter & Allen 252;* between Panamá and Chepo, *Dodge, Hunter, Steyermark & Allen 16654;* Arraiján, *Woodson, Allen & Seibert 1355;* Matías Hernández, *Pittier 6787;* Juan Díaz, *Standley 30626;* Río Tapía, *Standley 28085;* Alahuela, *Pittier 2328.* SAN BLAS: Puerto Obaldía, *Pittier 4285.*

Like all other species of *Heliconia, H. psittacorum* is highly variable, particularly in the pigmentation of the bracts and flowers. Segregation into varieties, if not species, may be feasible in the future.

ZINGIBERACEAE

Perennial herbs, usually aromatic, frequently of gigantic size, with horizontal tuberous rhizomes, caulescent or scapose; leaves spiral or distichous, with an open or closed basal ligulate sheath; inflorescence 1- to many-flowered, frequently cone-like and composed of rather showy bracts subtending the solitary or clustered flowers, terminating the leafy stem or on a special radical scape; flowers perfect, relatively small to very large and showy, usually strongly asymmetrical; perianth segments 6, in two series, the outer calyx-like, the inner petalaceous, more or less strongly united; fertile stamen 1, occasionally large and petalaceous, with a 2-celled anther; staminodia 1–3, the anterior (labellum) usually largest and frequently surpassing the corolla, thus imparting the aspect of an Orchid flower; ovary inferior, 2- to 3-celled; style terminal, usually elongate, free or more or less enveloped in a groove of the fertile stamen; stigma subcapitate; fruit fleshy and indehiscent or loculicidally 3-valved; seeds mostly accompanied by a fleshy aril.

Renealmia, Costus, and *Dimerocostus* are among the most conspicuous herbs of Panamanian thickets and bush at all but the highest elevations, although favoring the coastal region. The beauty of the flowers of several species is rivalled only by certain of the native Orchids. In addition to the three native genera, *Curcuma, Languas (Alpinia),* and *Hedychium* are cultivated frequently, as well as the ubiquitous Ginger *(Zingiber). Hedychium coronarium,* the Ginger Lily, with its large fragrant white flowers, has established itself as a particularly exuberant escape, usually in low rather marshy spots.

a. Leaves distichous, the sheaths open; flowers relatively small, the stamen not petaloid ..... 1. RENEALMIA
aa. Leaves spiral, the sheaths closed; flowers relatively larger, the stamen petaloid.
  b. Bracts longer than the fruit; bracteoles free, laminate; ovary 3-celled ..... 2. COSTUS
  bb. Bracts shorter than the fruit; bracteoles concrescent, tubular; ovary 2-celled ..... 3. DIMEROCOSTUS

1. RENEALMIA L. f.

Renealmia L. f. Suppl. 7. 1781.

Peperidium Lindl. Nat. Syst. 446. 1835.
Ethanium O. Ktze. Rev. Gen. 2:688. 1891

Rhizomatous herbs of mediocre to gigantic size; leaves distichous, with open ligulate sheaths; inflorescence paniculate, the branches scorpioid, rarely racemose, terminating the leafy stem, or scapose and arising directly from the rhizome; calyx tubular or turbinate, regularly 3-lobed or bursting irregularly at anthesis, usually more or less coriaceous or petalaceous; corolla tubular, 3-lobed; stamen not petalaceous; labellum more or less conspicuously 3-lobed; lateral staminodia absent or very inconspicuous; ovary inferior, 3-celled; ovules numerous; fruit a fleshy, irregularly dehiscent capsule.

a. Inflorescence terminating the leafy stem; bracts persistent in fruit, subcoriaceous ..... 1. R. cernua
aa. Inflorescence terminating a bracted scape arising directly from the rhizome; bracts deciduous in fruit, membranaceous.
b. Plants mediocre, 1–2 m. tall or less; flowers relatively small, the calyx 0.3–1.0 cm. long, persistent in fruit.
c. Inflorescences obviously paniculate, the bracts subtending few to several flowers.
d. Inflorescences thyrsiform, the bracts subtending an obvious secondary peduncle with few to several pedicellate flowers.
e. Calyx turbinate, with a spreading throat and a markedly tapered base.
f. Inflorescence relatively elongate and lax; leaves oblong-elliptic ..... 2. R. aromatica
ff. Inflorescence relatively short and congested; leaves oblanceolate ..... 3. R. mexicana
ee. Calyx urceolate, of about the same width throughout; inflorescence relatively elongate and lax; leaves oblanceolate ..... 4. R. concinna
dd. Inflorescence spiciform, the bracts subtending a fascicle of virtually sessile flowers, very congested and usually rather elongate; leaves obovate to oblanceolate; calyx turbinate ..... 5. R. costaricensis
cc. Inflorescences racemiform, the bracts subtending solitary flowers.
d. Leaves grass-like, 0.8–1.7 cm. broad; flowering scapes 7–10 cm. long ..... 6. R. Arundinaria
dd. Leaves obovate-elliptic, 3.5–7 cm. broad; flowering scapes 35–40 cm. long ..... 7. R. chiriquina
bb. Plants massive, 3–5 m. tall; flowers relatively large, the calyx 1.5–2.0 cm. long, usually circumscissile in fruit.
c. Flowering bracts membranaceous, oblong-lanceolate; corolla pink or red ..... 8. R. exaltata
cc. Flowering bracts subcoriaceous, oblong-ovate; corolla cream ..... 9. R. rubro-flava

1. Renealmia cernua (Sw.) Macbride in Field Mus. Publ. Bot. 11:14. 1931.

Costus cernuus Sw. ex R. & S. Syst. 1:25. 1817.
Renealmia strobilifera Poeppig & Endl. Nov. Gen. & Sp. 2:26. *pl. 136.* 1838.
Costus podocephalus Donn. Sm. in Bot. Gaz. 23:250. 1897.

Plants 1–3 m. tall; stems relatively slender; leaves oblong- to obovate-elliptic, 10–30 cm. long, 4–8 cm. broad, glabrous; inflorescence terminating the leafy

stem, sessile or subsessile, cone-like, ovoid to oblong-fusiform, 4–15 cm. long, the bracts persistent, oblong-lanceolate, acuminate to obtuse, 2–3 cm. long, orange or deep yellow tipped with yellow or green; corolla pale yellow, 0.7–1.0 cm. long; calyx acutely 3-lobed, 0.6–0.8 cm. long, persistent, somewhat accrescent in fruit; capsules broadly ovoid, about 1 cm. long.

Fig. 35. *Renealmia cernua*

Guatemala to Peru, in open forests chiefly at low elevations.

BOCAS DEL TORO: Changuinola Valley, *Dunlap 153;* Isla Colón, *von Wedel 27;* Fish Creek, *von Wedel 2320;* Old Bank Island, *von Wedel 1889;* Water Valley, *von Wedel 977.* CANAL ZONE: Río Puente, *Dodge & Allen 17475;* Río Trinidad, *Seibert 600;* Gatún, *Hayes 380;* Barro Colorado Island, *Aviles 29.* CHIRIQUÍ: San Bartolomé, *Woodson & Schery 877;* Puerto Armuelles, *Woodson & Schery 860.* COCLÉ: El Valle de Antón, *Woodson & Schery 172.* PANAMÁ: Río Tecúmen, *Standley 26731.*

Very much in evidence in the lowland forest, where it is apt to be mistaken by the newcomer for a species of *Costus* because of its colorful cone-like inflorescences.

2. RENEALMIA AROMATICA (Aubl.) Griseb. Fl. Brit. W. Ind. 601. 1864.

Alpinia aromatica Aubl. Hist. Pl. Guian. 1:3. 1775.
Alpinia multicaulis Aubl. loc. cit. 1775.
Alpinia occidentalis Sw. Prodr. Ind. Occ. 1:9. 1788.
Alpinia jamaicensis Gaertn. Fruct. et Sem. 1:36. *t. 12.* 1788.
Gethyra occidentalis (Sw.) Salisb. in Trans. Hort. Soc. Lond. 1:282. 1812.
Renealmia occidentalis (Sw.) Sweet, Hort. Brit. 493. 1830.

Leafy stems 1–2 m. tall; leaves oblong-elliptic, 20–30 cm. long, 3.5–8.0 cm. broad, shortly petiolate to subsessile; inflorescence scapose, arising directly from the rhizome, 4.5–6.0 dm. tall, the lower ⅔ sterile and bearing several leafless sheaths 3–8 cm. long, the flowering portion rather laxly but obviously paniculate, the branches bearing few to several rather small yellow flowers, densely puberulent; bracts oblong-lanceolate, 1–3 cm. long, membranaceous, deciduous; perianth 0.4–0.6 cm. long; calyx turbinate, 0.3–0.4 cm. long, persistent and accrescent in fruit; ovary narrowly ovoid, 0.2–0.3 cm. long, minutely puberulent; capsules scarlet or deep orange, broadly oblongoid, 0.7–0.8 cm. long.

Fig. 36. *Renealmia aromatica*

British Honduras to northern Brazil; Antilles; chiefly in lowland forest.

CANAL ZONE: Ancón Hill, *Killip 3049;* Barro Colorado Island, *Kenoyer 238.* COCLÉ: El Valle de Antón, *Allen 763.* PANAMÁ: between Balboa and Chamé, *Dodge, Hunter, Steyermark & Allen 16737;* Isla Taboga, *Woodson, Allen & Seibert 1463;* Matías Hernández, *Standley 28875.*

3. RENEALMIA MEXICANA Klotzsch ex Peters. in Mart. Fl. Bras. 3^3:45. 1890.

Leafy stems about 2 m. tall; leaves oblanceolate, abruptly acute or shortly acuminate at the tip, narrowly cuneate at the base, 25–40 cm. long, 8–12 cm. broad, distinctly petiolate; inflorescences scapose and arising directly from the rhizome, 20–30 cm. long, the lower ⅔ sterile and bearing several leafless sheaths 2–7 cm. long, the flowering portion very dense, paniculate, the branches bearing several flowers, densely puberulent; bracts oblong to oblong-obovate, 1.5–2.0 cm. long, deciduous, membranaceous; perianth 0.5–0.7 cm. long; calyx turbinate, 0.5–0.6 cm. long; ovary narrowly ovoid, 0.3–0.4 cm. long; capsules ellipsoid, about 1 cm. long.

Mexico to Panama, in lowland forest.

BOCAS DEL TORO: lower Changuinola River, *Stork 131;* Farm 6, vicinity of Almirante, *Rowlee & Stork 1012.*

These specimens may represent a phase of *R. costaricensis*, or possibly hybrids between that species and *R. aromatica.* It is odd that von Wedel did not encounter similar plants in his extensive collections about the Chiriquí Lagoon.

4. RENEALMIA CONCINNA Standl. in Jour. Wash. Acad. Sci. 17:249. 1927.

Leafy stems 3–6 dm. tall; leaves oblanceolate, abruptly acuminate at the tip, rather narrowly cuneate, 20–40 cm. long, 4–8 cm. broad, distinctly petiolate; inflorescence scapose and arising directly from the rhizome, 20–30 cm. long, the lower half sterile and bearing several leafless sheaths 1–3 cm. long, sparsely puberulent to glabrate, the flowering portion relatively slender and lax, subspiciform, the flowering branches short and bearing 2–4 rather small yellowish flowers; bracts obovate to ovate-lanceolate, 1.0–1.5 cm. long, membranaceous, deciduous; perianth 0.3–0.4 cm. long; calyx urceolate, about the same diameter throughout, 0.15–0.2 cm. long, green, the short lobes somewhat inflexed, accrescent in fruit; capsules broadly ellipsoid, about 0.8 cm. long.

Costa Rica and Panama, perhaps also in South America, in lowland forest and thickets.

BOCAS DEL TORO: Cricamola valley, *Cooper 196*. DARIÉN: Garagará, *Pittier 5599*.

I have a suspicion that this species may have an earlier name in the South American literature. It should be easily placed because of its distinctive calyx, but previous authors have paid little attention to this character in their descriptions.

5. RENEALMIA COSTARICENSIS Standl. in Field Mus. Publ. Bot. 18:190. 1937.
Renealmia densiflora Standl. in Jour. Wash. Acad. Sci. 17:249. 1927, non Urb.

Leafy stems 6–8 dm. tall, relatively stout; leaves obovate to broadly oblanceolate, obtuse or rounded to very abruptly acuminate, broadly cuneate at the base, 20–50 cm. long, 8–16 cm. broad, very shortly petiolate or subsessile; inflorescence scapose and arising directly from the rhizome, 20–40 cm. long, the lower ⅔ sterile, bearing several leafless sheaths 1.5–4.0 cm. long, minutely puberulent, the flowering portion very dense and compact, cylindrical, the bracts broadly obovate-suborbicular, 1.5–2.5 cm. long, green, tardily deciduous; perianth pale yellow, occasionally white, the labellum lemon-yellow, 0.5–0.6 cm. long; calyx turbinate, 0.3–0.4 cm. long, orange-pink, persistent and accrescent in fruit; capsules unknown at maturity.

Costa Rica and Panama, perhaps also in South America, in lowland thickets and open forest.

BOCAS DEL TORO: Río Cricamola, *Woodson, Allen & Seibert 1882*. COLÓN: Dos Bocas, *Pittier 4213*. SAN BLAS: Puerto Obaldía, *Pittier 4327*.

These plants recall the published description of *R. spicata* Gaignp. of the Guianas, specimens of which are not available for study at present.

6. RENEALMIA ARUNDINARIA Woodson in Ann. Missouri Bot. Gard. 29:329. 1942.

Flowering stem 3–4 dm. tall, relatively slender, glabrous throughout; leaves very narrowly oblong-lanceolate, grass-like, attenuate at both tip and base, 7–15 cm. long, 0.8–1.7 cm. broad, rather shortly petiolate; inflorescence scapose and arising directly from the rhizome, 7–10 cm. long, the lower ⅔ sterile and bearing several leafless sheaths about 1 cm. long, the flowering portion racemiform, relatively compact and dense, the bracts oblong-lanceolate, about 0.5 cm. long, subtending (apparently) each a solitary flower; perianth unknown; fruiting calyx narrowly turbinate, scarlet, 0.5 cm. long; capsules ellipsoid, 0.6–0.7 cm. long, scarlet.

Eastern Panama, probably also in Colombia, in lowland forest.

DARIÉN: Garagará, *Pittier 5597*.

7. RENEALMIA CHIRIQUINA Standl. in Field Mus. Publ. Bot. 22:7. 1940.

Flowering stems 4–8 dm. tall; leaves obovate-elliptic, 8–18 cm. long, 3.5–7.0 cm. broad; inflorescence scapose, arising directly from the rhizome, 35–40 cm. long, the sterile portion bearing rather distant leafless sheaths 2–5 cm. long, the flowering portion subcapitate, about 3 cm. long, bracts obovate-oblong, 1.0–1.5 cm. long, subtending solitary, light yellow or white flowers; perianth about 1 cm. long; calyx turbinate, 0.8–0.9 cm. long; capsule unknown.

Panama, in highland forest.

CHIRIQUÍ: Bajo Chorro, *Davidson 386;* Cerro Horqueta, *C. & W. von Hagen 210.*

A very peculiar plant of uncertain affinity.

8. RENEALMIA EXALTATA L. f. Suppl. 79. 1781.

Alpinia exaltata (L. f.) G. F. W. Meyer, Prim. Fl. Esseq. 4. 1818.
Alpinia Renealmia J. E. Sm. in Rees, Cycl. 39. no. 14. 1818.
Alpinia tubulata Lindl. in Bot. Reg. *pl. 777.* 1824.

Leafy stems very massive, 3–5 m. tall; leaves broadly lanceolate or obovate, 3–10 dm. long, 6–20 cm. broad, shortly petiolate or subsessile; inflorescence scapose, arising directly from the rhizome, 20–45 cm. long, the lower half sterile, bearing several large leafless sheaths 2–8 cm. long, the flowering portion paniculate, the branches bearing 2–3, rarely solitary, scarlet flowers of relatively large size; perianth 1.5–2.5 cm. long; calyx turbinate, 1.5–2.0 cm. long, scarlet, coriaceous, usually abscissing in fruit; capsules broadly ellipsoid, about 2 cm. long, deep purple.

Mexico to Brazil; Antilles. Chiefly in lowland forest and thickets.

BOCAS DEL TORO: Río Cricamola, *Woodson, Allen & Seibert 1905;* Almirante, *Skutch 8;* Water Valley, *von Wedel 983;* Western River, *von Wedel 2785.* COCLÉ: El Valle de Antón, *Allen 2151.* DARIÉN: Río Yape, *Allen 364.* CHIRIQUÍ: Bajo Mona and Quebrada Chiquero, *Woodson & Schery 601.*

9. RENEALMIA RUBRO-FLAVA K. Sch. in Engl. Pflanzenreich IV. 46:297. 1904.

Gigantic herb, the leafy stem attaining 5 m. in height; leaves broadly oblong-elliptic, shortly acuminate, broadly cuneate at the base, 6–8 dm. long, 20–25 cm. broad, sessile or subsessile; inflorescence scapose, arising directly from the rhizome, 25–30 cm. long (immature), the lower half sterile and densely covered with leafless sheaths 4–8 cm. long, the flowering portion paniculate, minutely puberulent, the branches bearing 2–4 rather mediocre cream-colored flowers; bracts subcoriaceous, broadly oblong, 3–7 cm. long; perianth about 2 cm. long; calyx turbinate, about 2 cm. long, persistent or circumscissile in fruit; capsule unknown.

Panama to Ecuador.

COCLÉ: El Valle de Antón, *Allen 1654.*

Perhaps only a variety of *R. exaltata.*

2. COSTUS L.

Costus L. Sp. Pl. 2. 1753.

Banksea Koenig, in Retz. Observ. 3:75. 1783.
Pyxa Noronha, Verh. Bat. Genootsch. 5^4:3. 1791.
Hellenia Retz. Observ. 6:18. 1791.
Tsiana Gmel. Syst. 9. 1791.
Planera Giseke. Prael. 205. 1792.
Glissanthe Salisb. Trans. Hort. Soc. Lond. 1:279. 1812.
Jacuanga Lestiboud. Ann. Sci. Nat. Bot. II. 15:329, 341. 1841.
Cadalvena Fenzl. Sitzungsber. Akad. Wien 51:139. 1863.

Rhizomatous perennial herbs of moderate to massive stature with unbranched stems (in Panama); leaves spiral, with closed, ligulate sheaths; inflorescence terminating the leafy stem (in Panama, elsewhere occasionally on special scapes directly from the rhizome), spiciform, cone-like, with conspicuous persistent imbricated bracts subtending one or few showy or rather inconspicuous flowers; calyx more or less equally 3-lobed, persistent; corolla somewhat unequally 3-lobed; stamen 1, conspicuously petalaceous; labellum (anterior staminodium) at least equalling and frequently far surpassing the corolla; ovary inferior, 3-celled, containing numerous ovules; fruit a tardily dehiscent, somewhat fleshy capsule.

a. All bracts with conspicuous foliaceous apical appendages.
  b. Bracts green or reddish within at the base; flowers 8–13 cm. long, the labellum very conspicuous, greatly surpassing the corolla and stamen.
    c. Plants very conspicuously ferruginous-hirsute; stems somewhat spiral, relatively slender; bracts lax and membranaceous, usually green within at anthesis; corolla pale yellow to nearly white; labellum yellow, usually without markings, widely spreading ... 1. C. VILLOSISSIMUS
    cc. Plants softly puberulent or pilosulose to essentially glabrous; stems straight and relatively stout; bracts turgid and subcoriaceous, usually reddish within at anthesis; corolla pale pinkish yellow to nearly white; labellum pinkish yellow with darker orange or yellow markings, sharply reflexed ... 2. C. FRIEDRICHSENII
  bb. Bracts deep red or pink throughout; flowers 3–5 cm. long, the labellum rather inconspicuous, about as long as the corolla and stamens; plants softly ferruginous-puberulent to nearly glabrous ... 3. C. LIMA
aa. Bracts without foliose apical appendages, or only the lowermost somewhat leaf-like.
  b. Flowers 7–10 cm. long, the labellum very conspicuous and spreading, reddish or purplish orange with showy pale yellow veins; plants massive; the stems stout and usually quite straight, the leaves narrowly elliptic to oblanceolate, the upper 30–40 cm. long ... 4. C. LAEVIS
  bb. Flowers 3–5 cm. long, the labellum essentially similar to the petals or somewhat less conspicuous, not venose; plants smaller, the stems relatively slender and usually somewhat spiral, the upper leaves 8–20 (rarely 30) cm. long.
    c. Bracts with the margins densely ciliate, otherwise minutely puberulent to glabrous, usually bright red with a conspicuous yellow callus, rarely orange or greenish; flowers red or reddish orange; leaves obovate or oblanceolate, gradually narrowed toward the base ... 5. C. RUBER
  cc. Bracts puberulent to glabrous, but the margins not ciliate.
    d. Bracts and flowers red; leaves broadly obovate-oval, rather obscurely subcordate-auriculate at the base; plants wholly glabrous ... 6. C. SPIRALIS

dd. Bracts and flowers yellow or orange, very rarely red; leaves obtuse or rounded at the base.
e. Bracts broadly obtuse or rounded throughout, with a more or less conspicuous linear callus; spikes relatively compact.
f. Leaves obovate to oblanceolate, broadest distinctly above the middle, densely ciliate on the margins, otherwise glabrous, the sheaths minutely puberulent to glabrous; spikes usually 4–5 cm. thick, cylindric or subcylindric and much longer than broad ..... 7. C. SPICATUS*
ff. Leaves elliptic, broadest at about the middle, generally puberulent beneath, particularly the midrib, rarely glabrate, the margins not ciliate, the sheaths rather sparsely spreading-villous, infrequently glabrate; spikes usually 1.5–2.5 cm. thick, broadly ovoid ..... 8. C. NUTANS
ee. Bracts rather narrowly acute, without a callus; spikes relatively loose, broadly ovoid, 4.5–5.0 cm. thick; leaves oblong-oblanceolate, broadest toward the tip, generally puberulent beneath, particularly the midrib, the sheaths puberulent or pilosulose ..... 9. C. SCABER*

*C. *spicatus* and *C. scaber* apparently hybridize with *C. nutans*.

1. COSTUS VILLOSISSIMUS Jacq. Fragm. Bot. 55. *pl. 80.* 1800-09.

Costus hirsutus Presl, Reliq. Haenk. 1:112. 1830.
Costus spicatus (Jacq.) Sw. var. *β. pubescens* Griseb. Fl. Brit. W. Ind. 602. 1864.

Plants rather stout, 2–3 m. tall, the stem somewhat spiral; leaves closely sheathing, obovate to broadly oblanceolate, apex abruptly acuminate, base broadly cuneate and very obscurely auriculate-subcordate, sessile or subsessile, the upper 15–30 cm. long, 5–13 cm. broad, very conspicuously ferruginous-pilose, particularly the sheaths, margins and midrib; inflorescence broadly ovoid, sessile and set amongst the upper somewhat reduced leaves, 5–10 cm. long; bracts all with spreading foliaceous tips, 5–8 cm. long, ferruginous-pilose, usually green within and without; calyx about 1 cm. long, broadly 3-dentate; ovary 0.5 cm. long; corolla pale yellow to nearly white, about 7 cm. long, the lobes oblong-elliptic; stamen 5–6 cm. long, oblong; labellum exceedingly showy, broadly 3-lobed, 9–10 cm. long, widely spreading, pale yellow, without markings (except in suspected hybrids with *C. Friedrichsenii*).

Guatemala to British Guiana and Peru, chiefly in lowland thickets and light forest.

CHIRIQUÍ: Remedios, *Woodson, Allen & Seibert 788.* COCLÉ: El Valle de Antón, *Allen 2185.* COLÓN: Cativa1, *Standley 30394.* CANAL ZONE: Ancón Hill, *Seibert 121;* Gold Creek, *Seibert 593;* Balboa, *Standley 29324;* Miraflores Lake, *G. White 186.* PANAMÁ: Campana, *Woodson, Allen & Seibert 1678;* Arraiján, *Woodson, Allen & Seibert 785;* Taboga Island, *Standley 27892;* Trapiche, Perlas Island, *Allen 2619.*

The delicately yellow orchidaceous flowers of this species lighten many a dingey lowland thicket in Panama. It apparently hybridizes with the equally frequent and even showier *C. Friedrichsenii.* In Panama, *C. villosissimus* is popularly known as *Cañagria* and *Caña de Mico,* and is said to be a remedy for venereal diseases.

Fig. 37. *Costus villosissimus*

2. Costus Friedrichsenii Peters. in Bot. Tidsskr. 18:260. 1893; Woodson in Ann. Missouri Bot. Gard. 29:329. 1942.

Plants stout, 2–4 m. tall, the stem stout and straight; leaves closely sheathing, broadly obovate-elliptic, rather shortly acuminate, rather broadly cuneate toward the base, 20–50 cm. long, 8–14 cm. broad, softly and densely puberulent beneath, somewhat scabrid above, the sheaths minutely and rather sparsely puberulent to glabrate; inflorescence broadly ovoid, sessile and set amongst the upper leaves, 12–15 cm. long; bracts all with rather turgid and recurved foliaceous tips, 5–9

Fig. 38. *Costus Friedrichsenii*

cm. long, very minutely puberulent to essentially glabrous, green but usually flushed with red within at anthesis; calyx about 1.3 cm. long, broadly 3-dentate; ovary about 0.7 cm. long; corolla pale pinkish yellow to nearly white, 7–8 cm. long, the lobes obovate-oblong; stamen 5–6 cm. long; labellum exceedingly showy, broadly 3- to 5-lobed, 10–11 cm. long, pinkish yellow with darker orange or reddish markings, sharply reflexed.

Guatemala to Bolivia, chiefly in lowland thickets and forest.

BOCAS DEL TORO: Little Bocas, *von Wedel 2520*. CANAL ZONE: Barro Colorado Island, *Standley 41068*; Ft. Randolph, *Maxon & Harvey 6510*. CHIRIQUÍ: Remedios, *Woodson, Allen & Seibert 789*. PANAMÁ: Arraiján, *Woodson, Allen & Seibert 1358*; Juán Díaz, *Stevens 1224*.

This is the most magnificent species of *Costus* in Panama. It has been confused until recently with *C. villosissimus*, although the two may be distinguished easily in or out of flower from quite a respectable distance.

3. COSTUS LIMA K. Sch. in Engl. Pflanzenreich IV. 46:388. 1904.

Costus lima K. Sch. var. *Wedelianus* Woodson in Ann. Missouri Bot. Gard. 26:277. 1939.

Plants stout, 2–4 m. tall, the stem somewhat spiral; leaves closely sheathing, oblong-elliptic, narrowly acuminate, broadly cuneate at the base, sessile or subsessile, 18–40 cm. long, 5–12 cm. broad, densely ferruginous-sericeous beneath, minutely scabrid above, the sheaths rather irregularly ferruginous-pilosulose; inflorescence broadly ovoid to rather narrowly cylindrical-fusiform, sessile and closely set amongst the upper somewhat reduced leaves; bracts deep red or pink throughout, with conspicuous acute reflexed apical appendages, ovate-lanceolate, 3–7 cm. long, minutely scabridulous; calyx about 0.8 cm. long, broadly 3-dentate; ovary 0.4 cm. long; corolla 3.0–5.0 cm. long, deep pink, the lobes obovate-oblong; labellum obovate-oblong, about equalling the corolla, pink; stamen 3 cm. long.

Costa Rica and Panama, possibly northward to Guatemala, in low thickets and open forest, particularly along swamps or rivers.

BOCAS DEL TORO: Nievecita, *Woodson, Allen & Seibert 1835;* Río Cricamola, *Woodson, Allen & Seibert 1926;* Isla Colón, *von Wedel 28;* Water Valley, *von Wedel 1632.* CHIRIQUÍ: Remedios, *Woodson, Allen & Seibert 786.* COCLÉ: Penonomé, *Williams 431.*

Very conspicuous because of the inflorescences like bloody pikes. *C. Bakeri* K. Sch., of Guatemala, possibly is synonymous.

4. COSTUS LAEVIS R. & P. Fl. Peruv. 1:3. 1798; Woodson in Ann. Missouri Bot. Gard. 29:330. 1942.

Costus laxus Peters. in Mart. Fl. Bras. 3^3:56. 1890.
Costus giganteus O. Ktze. Rev. Gen. 2:687. 1891, non Ridl.
Costus splendens Donn. Sm. & Tuerckh. in Bot. Gaz. 33:260. 1902.
Costus maximus K. Sch. in Engl. Pflanzenreich IV. 46:405. 1904.
Costus Weberbaueri Loesn. in Notizblatt. K. Bot. Gart. 10:712. 1929.
Costus Malortieanus Wendl. var. *amazonicus* Loesn. loc. cit. 710. 1929.
Costus amazonicus (Loesn.) Macbr. in Field Mus. Publ. Bot. 11:13. 1931.
Costus Skutchii Morton in Jour. Wash. Acad. Sci. 27:306. 1937.

Plants usually quite stout, 1–4 m. tall, essentially glabrous throughout, or the bracts and leaves indefinitely papillate; stems straight or somewhat spiral; leaves narrowly elliptic to oblanceolate, acuminate, broadly cuneate and obscurely subauriculate at the base, shortly subpetiolate, 15–40 cm. long, 4–12 cm. broad; inflorescence broadly ovoid to oblong-fusiform, sessile and set closely amongst the upper reduced leaves, 5–25 cm. long; bracts without appendages or only the lowermost somewhat leaf-like, broadly ovate, obtuse, 2.5–3.5 cm. broad, deep yellowish green flushed with crimson within at anthesis, usually with a conspicuous yellow linear callus; calyx 1 cm. long, broadly 3-dentate; ovary 0.4 cm.

Fig. 39. *Costus laevis*

long; corolla 4.0–6.5 cm. long, yellowish white, the lobes obovate-oblong; labellum 7–8 cm. long, broadly 3-lobed and sharply reflexed, reddish orange with pale yellow reticulations.

Guatemala to Peru and northern Brazil, chiefly in lowland thickets and open forest.

BOCAS DEL TORO: Río Cricamola, *Woodson, Allen & Seibert 1929;* Water Valley, *von Wedel 1507;* Little Bocas, *von Wedel 2506;* Fish Creek, *von Wedel 2219a.* CANAL ZONE: Ft. Randolph, *Standley 28672;* Chagres, *Fendler 447;* Quebrada Ancha, *Steyermark & Allen 17112.* COCLÉ: El Valle de Antón, *Allen 2199.*

A magnificent species outstanding in the genus because of the showy flowers with reticulated labellum.

5. COSTUS RUBER Griseb. Cat. Pl. Cub. 256. 1866; Woodson in Ann. Missouri Bot. Gard. 29:330. 1942.

Costus formosus Morton in Jour. Wash. Acad. Sci. 27:305. 1937.
Costus spicatus of authors, not Jacq.
Costus spiralis of authors, not Rosc.

Plants 0.5–3.0 m. tall, the stem strongly spiral; leaves obovate to oblanceolate, very abruptly acuminate, broadly cuneate at the base, 8–30 cm. long, 4–10 cm. broad, glabrous or essentially so, sessile or subsessile; inflorescence ellipsoid to narrowly fusiform, set rather closely amongst the upper reduced leaves, 3–16 cm. long, usually sharply pointed at the tip; bracts broadly ovate, acute, unappendaged, 2.0–3.5 cm. long, usually bright red with a conspicuous yellow callus, rarely orange or greenish, densely ciliate with short more or less arachnoid hairs, otherwise minutely puberulent-papillate to glabrous; calyx about 0.7 cm. long, broadly 3-dentate, deep pink; ovary 0.3 cm. long; corolla 3–4 cm. long, scarlet to pinkish orange, the lobes obovate-oblong; labellum 4–5 cm. long, obovate-oblong, scarlet to reddish orange; stamen 5–6 cm. long, rather narrowly oblong.

Guatemala to Colombia; Antilles. Chiefly in lowland thickets and open forests.

BOCAS DEL TORO: Pumpkin River, *von Wedel 2586.* CANAL ZONE: Gold Creek, *Seibert 584;* Mojinga Swamp, *Allen 866;* Barro Colorado Island, *Woodson & Schery 966;* Río Pequení, *Woodson, Allen & Seibert 1597;* Quebrada Ancha, *Steyermark & Allen 17112.* CHIRIQUÍ: Puerto Armuelles, *Woodson & Schery 857;* San Felix, *Allen 1956;* San Bartolomé, *Woodson & Schery 886.* COCLÉ: El Valle de Antón, *Seibert 419, Allen 1825.* DARIÉN: Pinogana, *Allen 938.*

This is probably the most attractive of the smaller species of *Costus. C. sanguineus* Donn. Sm. of Guatemala possibly is synonymous.

6. COSTUS SPIRALIS (Jacq.) Rosc. Monandr. Pl. *pl.* [*79*]. 1828; Woodson in Ann. Missouri Bot. Gard. 29:331. 1942.

Alpinia spiralis Jacq. Hort. Schoenbr. 1:1. *tab. 1.* 1797.
Costus Pisonis Lindl. in Bot. Reg. *pl. 899.* 1825.

Fig. 40. *Costus ruber*

Plants 1–2 m. tall, essentially glabrous throughout, the stem somewhat spiral; leaves broadly obovate-oval, abruptly subcaudate-acuminate, base rather obscurely subcordate-auriculate, 12–25 cm. long, 4–8 cm. broad, very shortly petiolate; inflorescence closely set amongst the upper reduced leaves, very broadly ovoid, 2–5 cm. long; bracts unappendaged, broadly ovate, 1.5–2.0 cm. long, deep red, gla-

brous; calyx 0.5–0.6 cm. long, broadly 3-dentate, red; ovary 0.4 cm. long; corolla 2.0–2.5 cm. long, deep red, the stamen and labellum about equal.

Antilles; Panama, probably also in Costa Rica and northern Colombia. in lowland thickets.

BOCAS DEL TORO: Isla Colón, *von Wedel 2939;* Old Bank Island, *von Wedel 2000;* Bastimentos Island, *von Wedel 2899.*

This species appears to be limited to the islands of the Chiriquí Lagoon. The specimens coincide strikingly with Jacquin's illustrations of *C. spiralis,* and particularly with those of Roscoe.

7. COSTUS SPICATUS (Jacq.) Sw. Prodr. Ind. Occ. 11. 1788.

Alpinia spicata Jacq. Select. Stirp. Amer. Hist. *pl. 1.* 1763.
Costus conicus Stokes, Mat. Med. 1:75. 1812.

Plants relatively slender, 1–3 m. tall, the stems somewhat spiral; leaves obovate to oblanceolate, narrowly and usually rather abruptly acuminate, base cuneate, 9–30 cm. long, 3–12 cm. broad, glaucous, the margins ferruginous-ciliolate, otherwise glabrous, very shortly petiolate; inflorescence sessile, set amongst the upper reduced leaves, broadly ovoid to cylindrical, 5–14 cm. long; bracts unappendaged or the lowermost somewhat leaf-like, broadly ovate, obtuse, 2–3 cm. long, orange with a yellowish linear callus, glabrous or indefinitely papillate; calyx 0.5 cm. long, broadly 3-dentate, pale yellow flushed with pink at the tips; ovary 0.4 cm. long; corolla 2–3 cm. long, orange-yellow, the lobes obovate-oblong; labellum 3.0–3.5 cm. long, yellow; stamen about equalling the labellum.

Antilles; Costa Rica to Caribbean Colombia, chiefly in lowland thickets and open forest.

BOCAS DEL TORO: Nievecita, *Woodson, Allen & Seibert 1951.* CHIRIQUÍ: San Bartolomé, *Woodson & Schery 922;* Quebrada Velo, *Woodson & Schery 282.*

Great uncertainty has attended the application of *C. spicatus, C. cylindricus,* and *C. spiralis.* The plants cited above coincide well with authoritative icones, but, because of the current confusion of names, it is difficult to assign the exact range of the species. *C. spicatus* apparently hybridizes with *C. nutans* in the Pacific foothills *(Woodson & Schery 282).*

8. COSTUS NUTANS K. Sch. in Engl. Pflanzenreich IV. 46:407. 1904.

Slender plants about 1 m. tall; stems strongly spiral; leaves elliptic, narrowly acuminate or subcaudate-acuminate, base broadly acute, 7–14 cm. long, 2–6 cm. broad, very shortly petiolate, rather inconspicuously ferruginous-pilose beneath, glabrous above or the midrib ferruginous-pilosulose; sheaths ferruginous-pilose, particularly above; inflorescence ovoid, rather sharp-pointed, set closely amongst

the upper leaves, 3–5 cm. long; bracts broadly ovate, obtuse, unappendaged, 1.5–2.0 cm. long, bright yellow to orange, rarely scarlet, with a rather inconspicuous yellow callus; calyx 0.3 cm. long, broadly 3-dentate; ovary 0.3 cm. long; corolla about 2 cm. long, yellow, about equalling the labellum and stamen.

Costa Rica and Panama, probably also in northwestern Colombia, in mountain and foothill forests and thickets.

CHIRIQUÍ: Boquete, *Woodson, Allen & Seibert 1169;* San Felix, *Allen 1952.* COCLÉ: El Valle de Antón, *Seibert 454, Allen 1789;* Cerro Valle Chiquito, *Seibert 508;* Las Margaritas, *Woodson, Allen & Seibert 1231.* DARIÉN: Mt. Pirrí, *Goldman 1963.*

C. nutans apparently hybridizes with *C. scaber,* and the following plants probably should be construed as evidence: CHIRIQUÍ: Bajo Mona, *Woodson, Allen & Seibert 1000;* Boquete, *Davidson 726;* Río Gariché, *Seibert 330.* Although these specimens should key to *C. nutans,* their relationship to *scaber* is indicated by their larger stature, their more massive inflorescences, and their lack, or virtual lack, of the ferruginous pilosity of *C. nutans.*

9. COSTUS SCABER R. & P. Fl. Peruv. 1:2. *pl. 3.* 1798; Woodson in Ann. Missouri Bot. Gard. 29:330. 1942.

Rather stout plants 1–2 m. tall, the stem somewhat spiral, usually disproportionally thick; leaves oblong-oblanceolate, rather abruptly acuminate, broadly cuneate toward the base, very shortly petiolate, 15–30 cm. long, 5–8 cm. broad, generally puberulent beneath, particularly the midrib, essentially glabrous above; inflorescence broadly ovoid, set amongst the reduced upper leaves, 3–8 cm. long; bracts ovate, rather narrowly acute, 2.5–4.0 cm. long, orange to scarlet, without a callus, essentially glabrous; calyx about 1 cm. long, broadly 3-dentate, red; ovary 0.4 cm. long; corolla about 3 cm. long, the labellum and stamen about equalling, bright yellow.

Panama to Peru, probably also in Costa Rica, in mountain and foothill thickets and open forest.

CHIRIQUÍ: Bajo Mona, *Woodson & Schery 544.* COCLÉ: El Valle de Antón, *Woodson & Schery 202.* PANAMÁ: Campana, *Allen 1873.*

3. DIMEROCOSTUS O. Ktze.

DIMEROCOSTUS O. Ktze. Rev. Gen. 2:687. 1891.

Gigantic rhizomatous unbranched herbs; leaves spiral, with closed ligulate sheaths, congested near the tip of the elongate stem; inflorescence spiciform, scarcely cone-like as in *Costus,* the bracts much shorter than the subtended clusters of flowers, the bracteoles concrescent and tubular, closely investing the ovaries; calyx tubular, 3-parted above; corolla 3-lobed; labellum extremely showy; stamen 1, petaloid; ovary inferior, 2-celled; fruit a tardily dehiscent capsule.

1. DIMEROCOSTUS UNIFLORUS (Poeppig) K. Sch. in Engl. Pflanzenreich IV. 46:427. 1904.

Costus uniflorus Poeppig. ex Peters. in Mart. Fl. Bras. 3^3:58. 1890.
Dimerocostus strobilaceus O. Ktze. Rev. Gen. 2:687. 1891.

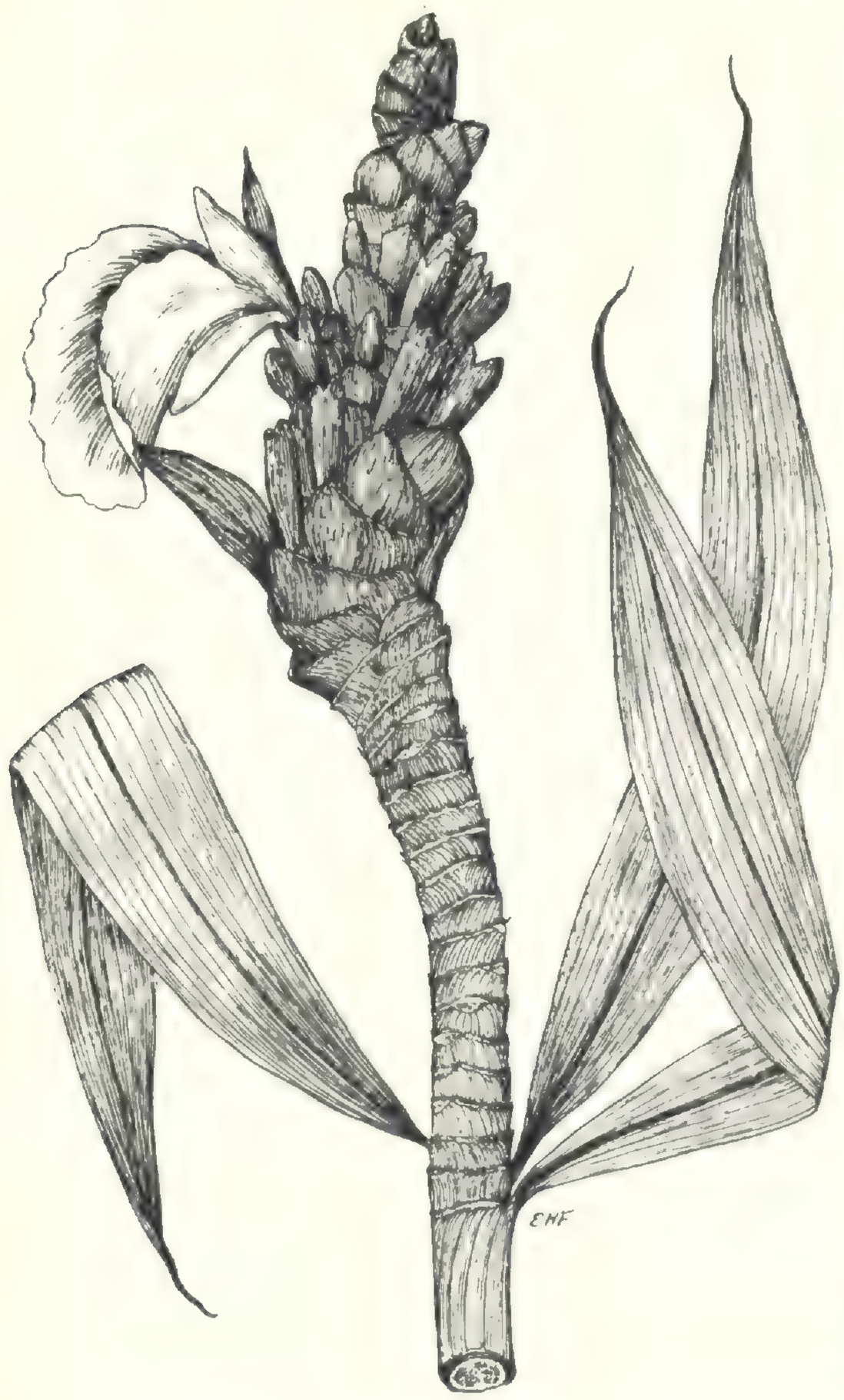

Fig. 41. *Dimerocostus uniflorus*

Plants very stout, 3–6 m. tall; stem somewhat spiral; leaves congested toward the top of the stem, oblong-oblanceolate, narrowly acuminate, gradually narrowed to the base, sessile, 20–45 cm. long, 5–7 cm. broad, minutely sericeous to essentially glabrous beneath; inflorescence cylindrical, somewhat spirally contorted, 15–30 cm. long, 5–6 cm. broad, bearing numerous closely sheathing green closed bracts 2–3 cm. long, each usually with a linear callus; calyx 2–3 cm. long, coriaceous, ovary subcylindrical, 2 cm. long, both minutely sericeous; corolla white, or yellowish within, 7–8 cm. long, the lobes narrowly oblong; labellum extremely showy, broadly 2-lobed, 9–11 cm. long and broad, white; stamen 3–4 cm. long.

Costa Rica to Peru, in lowland thickets and open forests.

BOCAS DEL TORO: Isla Colón, *von Wedel 25;* Water Valley, *von Wedel 2664.* CANAL ZONE: Río Pequení, *Allen 17274;* Las Cruces, *Seibert 582;* Barro Colorado Island, *Dodge 3461;* Ft. San Lorenzo, *Maxon & Valentine 6990;* Colón, *Kuntze 1873.* DARIÉN: Marragantí, *Williams 689.* PANAMÁ: Hacienda La Joya, *Dodge, Hunter, Steyermark & Allen 16915;* Río Tapia, *Standley 28127.*

The elongate unbranched stems of *D. uniflorus,* with their crowns of elongate leaves and their magnificent white flowers which open only one at a time upon any plant, create a most striking and familiar sight in lowland Panama.

CANNACEAE

1. CANNA L.

CANNA L. Sp. Pl. 1. 1753.

Katubala Adans. Fam. 2:67. 1763.
Cannacorus Tourn. ex Medic. in Acta Acad. Theod.-palat. 6: Phys. 378. 1790.
Xyphostylis Raf. Fl. Tellur. 4:52. 1836.
Distemon Bouché in Linnaea 18:494. 1844.
Eurystylus Bouché, loc. cit. 485. 1844.

Mediocre to fairly massive, leafy, rhizomatous herbs; leaves spiral, relatively large, with an eligulate sheath; inflorescence racemiform or paniculate, bracteate, the bracts usually subtending a cicinnus of 2 more or less showy, usually brightly colored perfect flowers; sepals 3, free, more or less foliaceous or petalaceous, essentially equal; petals 3, nearly equal, more or less connate at the base; fertile stamen 1, petaloid, bearing a solitary marginal anther, more or less connate at the base with the somewhat petaloid style, a petaloid anterior (labellum) and 2–3 showy posterior staminodia; ovary inferior, 3-celled, conspicuously warty or spiny-fimbriate, containing numerous ovules; fruit a rather large warty or spiny-fimbriate capsule finally opening by the collapse of the pericarp; seeds round and very hard.

The taxonomy of the Cannas is about as troublesome as that of any of the Monocotyledons, not because of the original number of species probably, but because they have been cultivated and hybridized since the earliest years of European colonization of America. Pressed specimens do not give a good idea of the habit of the plants, nor of the aspect and color of the flowers and foliage. Early systematic studies of the genus were undertaken, however painstakingly by such enthusiasts as Roscoe, under conditions of greenhouse and garden culture in Europe, and the numerous species proposed mostly upon single specimens of doubtful origin. Most herbaria contain far more specimens of garden hybrid Cannas than of undoubtedly indigenous plants accompanied by adequate data. Such genera are not attractive subjects for professional taxonomists, and as a result *Canna* has suffered from neglect as well as from misunderstanding. Such accounts as that of Kränzlin (in Engl. Pflanzenreich IV. 47. 1912) are obviously pieces of chore-work guided by little biological understanding of the problem, and by no real interest in it.

Under such circumstances, preparation of an account of *Canna* for a Flora such as this is very difficult. Because of our interest in the Scitamineae of Panama generally, the editors have devoted considerable attention to the species of *Canna*, in the field, in the herbarium, and in the library. We also have had the opportunity of examining numerous exsiccatae annotated by Kränzlin, which, however, have been of little actual use since they so frequently fail to coincide with our information from other sources. The following account, therefore, is virtually

independent, and although the definition of the biological entities is probably as satisfactory as may be obtained at present, the nomenclature undoubtedly will bear scrutiny in the light of future monographic study.

In Panama, *Canna* is not a particularly prominent element of the herbaceous flora since the plants do not grow in such conspicuous stands as do various Heliconias and Calatheas, for example. Their showy flowers, nevertheless, have earned them popular recognition as *Plantanillo* (generally applied to any plant at all resembling a Banana), and *Café cimarron* or *Café silvestre* (because of their hard round seeds). The garden hybrids frequently encountered are known as *Bandera española.* The West Indians call the seeds "Indian shot", and they are occasionally used in boys' popguns. The leaves or roots are reported as used in some districts as domestic medicine for diuretics and emollient poultices. The large leaves, like those of Heliconias and Calatheas, are employed in the "interior" for wrapping small parcels.

a. Flowers 4–10 cm. long, yellow, red, or variegated; plants with the true habit of a *Canna*, the inflorescence vertical.
  b. Staminodia 2; flowers yellow, usually spotted with orange or red, 5–6 cm. long; leaves broadly ovate to ovate-lanceolate, the base broadly obtuse or rounded, then abruptly decurrent to the petiole ... 1. C. LUTEA
 bb. Staminodia 3.
  c. Leaves lanceolate to elliptic-lanceolate, gradually and continuously narrowed to the petiole, glaucous; flowers yellow.
   d. Flowers 8–9 cm. long; corolla tube about as long as the calyx, the lobes erect or strongly ascending; leaves narrowly lanceolate ... 2. C. GLAUCA
   dd. Flowers 9–10 cm. long; corolla tube greatly surpassing the calyx, the lobes reflexed; leaves elliptic-lanceolate ... 3. C. FLACCIDA
  cc. Leaves ovate to ovate-elliptic, obtuse or rounded to broadly acute at the base, then abruptly decurrent to the petiole, not glaucous or scarcely so; flowers red, occasionally flushed with yellow at the base.
   d. Flowers 4–5 cm. long, shortly pedicellate or subsessile; corolla lobes spreading ... 4. C. INDICA
   dd. Flowers 6–8 cm. long, the pedicels as long as the ovary or longer; corolla lobes nearly erect ... 5. C. EDULIS
aa. Flowers 10–12 cm. long, white tinged with yellowish green at the tips; plants with the habit of a *Heliconia*, the inflorescence horizontal ... 6. C. LILIIFLORA

1. CANNA LUTEA Mill. Gard. Dict. ed. 8, no. 4. 1768.

Canna aurantiaca Rosc. Monandr. Pl. *pl.* [*21*]. 1828.
Canna maculata Link, Handb. 1:227. 1829.
Canna commutata Bouché in Linnaea 8:147. 1833.
Canna densiflora Bouché, loc. cit. 18:489. 1844.
Canna floribunda Bouché, loc. cit. 1844.

Plants 1.0–1.5 m. tall, glabrous throughout; leaves broadly ovate to ovate-lanceolate, acute to very shortly acuminate, the base broadly obtuse or rounded then abruptly and shortly decurrent to the petiole (sheath), 20–45 cm. long, 10–25 cm. broad; inflorescence erect, racemiform or divided at the base, bearing several or rather few very shortly pedicellate or sessile, solitary or paired flowers; sepals oblong, 0.5–0.7 cm. long; corolla 3.0–3.5 cm. long, the lobes narrowly

oblong-lanceolate, acuminate, the tube about 0.5 cm. long, yellow; staminodia 2, oblanceolate, 5–6 cm. long, yellow spotted with orange or red; capsules oblong-subclavate, 3.0–3.5 cm. long, densely muricate.

Mexico to southern Brazil; Antilles. In wet thickets and open forest, chiefly at fairly low elevations.

CANAL ZONE: Culebra, *Pittier 2525;* Las Cascadas, *Standley 29689.* COCLÉ: Cerro Valle Chiquito, *Seibert 500;* Las Margaritas, *Woodson, Allen & Seibert 1233.*

2. CANNA GLAUCA L. Sp. Pl. 1. 1753.

Canna angustifolia L. Sp. Pl. 1:1. 1767.
Canna stricta Bouché in Linnaea 12:144. 1838.
Canna liturata Link ex Dietr. Syn. Pl. 1:12. 1839.
Canna Schlechtendaliana Bouché, loc. cit. 18:487. 1844.

Plants 1.5–2.0 m. tall; leaves rather narrowly lanceolate, acuminate, the base gradually and continually narrowed to the sheath, 30–45 cm. long, 8–15 cm. broad, glabrous and conspicuously glaucous; inflorescence racemiform, simple, bearing several pairs of shortly pedicellate or subsessile flowers; sepals oblong-elliptic, about 1 cm. long; corolla 4.0–4.5 cm. long, yellow, the lobes oblong-lanceolate, acuminate, erect or sharply ascending, the tube about equalling the sepals; staminodia 3, obovate-elliptic, 8–9 cm. long, yellow; capsules irregularly ellipsoid, 4–5 cm. long, 2–3 cm. thick, densely spiny-fimbriate.

Panama to the Guianas and Argentina; Antilles, in lowland thickets and open forest.

BOCAS DEL TORO: Old Bank Island, *von Wedel 2001.*

3. CANNA FLACCIDA Salisb. Icon. Stirp. Rar. 3. *pl. 2.* 1791.

Canna flava Michx. ex Lam. in Jour. Nat. Hist. Par. 1:416. 1792.
Canna elegans Raf. Fl. Ludovic. 143. 1817.
Eurystylus flaccida Bouché in Linnaea 18:485. 1844.

Plants 1–2 m. tall, wholly glabrous; leaves elliptic-lanceolate, narrowly acute to acuminate, base gradually and continuously narrowed to the sheath, 20–45 cm. long, 8–11 cm. broad, glaucous; inflorescence racemiform, simple, bearing a few pairs of virtually sessile, showy yellow flowers; sepals oblong-elliptic, 2–3 cm. long; corolla 8–9 cm. long, yellow, the lobes narrowly oblong-lanceolate, sharply reflexed at anthesis, the tube 3–4 cm. long; staminodia broadly obovate, 9–10 cm. long, yellow; capsules irregularly ellipsoid, 5–6 cm. long, 4.0–4.5 cm. thick, spiny-fimbriate.

Coastal plain of South Carolina, Georgia, and Florida; Antilles; Panama. In lowland thickets.

BOCAS DEL TORO: Isla Colón, *von Wedel 78, 89.* CANAL ZONE: Rio Pedro Miguel, in garden, *Standley 30015.*

Fig. 42. *Canna flaccida*

4. CANNA INDICA L. Sp. Pl. 1. 1753.

Canna indica var. *patens* Ait. Hort. Kew. 1:1. 1789.
Cannacorus indicus Medic. in Acta Acad. Theod.-palat. 6: Phys. 379. 1790.
Cannacorus ovatus Moench, Meth. 526. 1794.
Canna patens (Ait.) Rosc. Trans. Linn. Soc. 8:338. 1807.
Canna sylvestris Rosc. acc. to Kränzl. in Engl. Pflanzenr. IV. 47:61. 1912, as to Panamanian exsiccatae.

Canna Warscewiczii A. Dietr. acc. to Kränzl. loc. cit. 64. 1912, as to Panamanian exsiccatae.

Plants 1–3 m. tall, glabrous throughout; leaves ovate to ovate-elliptic, acute to shortly acuminate, base broadly obtuse or rounded then abruptly and shortly decurrent to the sheath, 15–50 cm. long, 9–20 cm. broad; inflorescence usually branched several times toward the base, bearing numerous paired, shortly pedicellate to subsessile flowers; sepals lanceolate, 0.9–1.2 cm. long; corolla 3–4 cm. long, red frequently flushed with yellow toward the base, the lobes narrowly oblong-lanceolate, narrowly acuminate, spreading, the tube about as long as the sepals; staminodia oblanceolate, 4–5 cm. long, red frequently flushed with yellow or orange at the base; capsules irregularly ellipsoid, 2–4 cm. long, 1.5–2.5 cm. broad, densely spinose-muricate.

Mexico to southern Brazil; Antilles. Chiefly in lowland thickets and open forest.

BOCAS DEL TORO: Almirante, *Skutch 13;* Talamanca valley, *Carleton 123.* CANAL ZONE: Culebra, *Pittier 2524;* Balboa, *Standley 25551, 26070.* DARIÉN: Boca de Cupe, *Williams 820.* PANAMÁ: Juan Díaz, *Standley 30486;* Panamá, *Maxon, Harvey & Valentine 7097.*

Although annotated and cited by Kränzlin as representing two distinct species, neither *C. indica,* I cannot reconcile myself to consider these specimens other than a single biological entity. In the absence of more acceptable authority, I am assigning them to *C. indica,* the plates of which, provided by Roscoe, agree sufficiently well with our plants. *C. coccinea* Mill. probably also should be added as a synonym.

5. CANNA EDULIS Ker in Bot. Reg. *pl. 775.* 1823.

Canna indica R. & P. Fl. Peruv. 1:1. 1798, non L.

Plants 2–3 m. tall, glabrous throughout; leaves ovate-elliptic, acute to acuminate, base broadly obtuse then abruptly and shortly decurrent to the sheath, 30–65 cm. long, 15–20 cm. broad; inflorescence divided once or twice from the base, bearing numerous pairs of rather long-pedicellate flowers; sepals 1.0–1.2 cm. long; ovary clavate, 0.8–0.9 cm. long; corolla 4.5–5.0 cm. long, the lobes oblong-elliptic, erect or nearly so, the tube shorter than the sepals, bright red to orange; staminodia oblong-oblanceolate, 6–8 cm. long, free nearly to the base, red; capsules broadly ellipsoid, about 5 cm. long, 2.0–2.5 cm. thick, densely spinose-muricate.

Panama to Peru, Bolivia, and northern Argentina, in moist highland forest.

CHIRIQUÍ: Boquete, *Davidson 500, Pittier 2979;* Río Chiriquí Viejo valley, *Seibert 295, Allen 1389.*

6. CANNA LILIIFLORA Warsc. ex Planch. in Fl. Serres 10:211. *pl. 1055-1056.* 1854; Kränzl. in Engl. Pflanzenreich IV. 47:70. 1912.

Fig. 43. *Canna edulis*

Plants 2.5–3.0 m. tall, wholly glabrous; leaves subhorizontal, oblong, acuminate, 90–120 cm. long, about 45 cm. broad; inflorescence simple or infrequently branched, horizontally deflexed, bracteate, the bracts about 4 cm. long, subtending solitary, exceedingly showy flowers; sepals oblong, longer than the ovary, green; corolla white, 12–14 cm. long, the lobes oblong-linear, 2 cm. broad; staminodia 3, subequal, apparently distinctly longer than the corolla lobes, about 2.5 cm. broad.

Fig. 44. *Canna liliiflora* (redrawn from Engler)

This description is adapted from that provided by Kränzlin. *Canna liliiflora* apparently was collected but once, by Warscewicz in "Veraguas", a rough designation of earlier days for the whole western half of Panama. Although expeditions from the Missouri Botanical Garden have been on the lookout for it for several years, no trace of it has been found. A truly white Canna of such magnificent stature would be a horticultural rediscovery of real distinction.

MARANTACEAE

Rhizomatous perennial herbs, caulescent or acaulescent, frequently massive; leaves radical or cauline, usually 2-ranked, differentiated into a blade, petiole, and sheath, the petiole hardened and callous at least in part; inflorescence terminating the stem, or scapose and arising directly from the rhizome, spiciform or paniculate, infrequently diffusely cymose, the bracts deciduous or persistent, frequently fairly large and colored and imparting a cone-like aspect to the inflorescence; flowers perfect, very asymmetric, epigynous; sepals 3, free, essentially equal; corolla 3-parted, the lobes connate toward the base, unequal; fertile stamen 1, petaloid, bearing a marginal anther; staminodia 1–5, more or less petaloid, one usually forming a hood about the style; ovary inferior, 3- to 1-celled, sometimes 2 of the cells sterile; fruit a loculicidal capsule, rarely fleshy and indehiscent; seeds provided with a more or less conspicuous aril.

The Marantaceae form a conspicuous element of the herbaceous flora of Panama, particularly in low thickets and marshes. In these places large stands of such species as *Calathea lutea,* with their large broad leaves standing nearly upright and the waxy lower surface directed outward, create a very striking effect. The leaves of several species are used as wrapping paper, frequently for articles of food, as tamales and cakes of native sugar, *carpadurra.* The tuberous rhizomes of *Maranta arundinacea,* known as *Sagú,* yield arrowroot laundry starch.

The Marantaceae have not been spared the general taxonomic neglect accorded the Scitaminales as a whole. In the case of this family, however, confusion does not exist as much in specific as in generic distinctions. Schumann's account of the family for Engler's 'Pflanzenreich' has followed Eichler in a number of very difficult generic segregations, which, in the absence of a contemporary authority, have been ignored in this treatment for the 'Flora of Panama' (cf. Woodson & Schery, Ann. Missouri Bot. Gard. 29:331-335. 1942).

a. Ovary 3-celled; fruit 3-seeded ..... 1. CALATHEA
aa. Ovary 1-celled; fruit 1-seeded.
  b. Bracts persistent; rachis closely articulated but scarcely zig-zag.
    c. Bracts not closely imbricated at anthesis; corolla tube very short; outer staminodia 2, creamy white ..... 2. MYROSMA
    cc. Bracts closely imbricated at anthesis; corolla tube narrow and elongate; outer staminodium 1, white or purple ..... 3. ISCHNOSIPHON
  bb. Bracts caducous.
    c. Inflorescence lax and relatively few-flowered, the rachis rather distantly flexuose; bracts green; outer staminodia 2, white, very conspicuous and labelliform; fruits nut-like, indehiscent ..... 4. MARANTA
    cc. Inflorescence congested and very many-flowered, the rachis closely and sharply zig-zag.
      d. Bracts orange or yellow; outer staminodia 2, white or yellowish, subequal; fruits capsular, dehiscent ..... 5. STROMANTHE
      dd. Bracts green; outer staminodium 1, purple; fruits nut-like, indehiscent ..... 6. THALIA

1. CALATHEA G. F. W. Meyer

Calathea G. F. W. Meyer, Prim. Fl. Esseq. 6. 1818.

Endocodon Raf. Fl. Tellur. 4:49. 1836.
Zelmira Raf. loc. cit. 50. 1836.
Monostiche Koernicke in Gartenfl. 7:76, 88. 1858.

Massive to relatively small perennial herbs, caulescent or acaulescent; leaves 2-ranked, less frequently spiral; inflorescence terminating the stem or arising directly from the rhizome, spiciform, simple, solitary or clustered, the bracts usually closely imbricated, persistent, foliaceous to chartaceous or coriaceous and frequently strikingly colored, subtending cicinnate clusters of rather small subsessile, bracteolate flowers; flowers perfect, very asymmetric, epigynous; sepals 3, more or less connate at the base, the lobes essentially equal; corolla lobes 3, unequal, connate at the base into a fairly short tube; fertile stamen 1, petaloid, united at the base with 2–3 more or less conspicuous petaloid staminodia; ovary inferior, 3-celled; fruit a loculicidally dehiscent, 3-seeded capsule.

a. Bracts 2-ranked.
  b. Spikes borne upon leafy stems; bracts numerous, closely imbricated.
    c. Spikes relatively narrow, the bracts sharply ascending and nearly parallel to the peduncle, heavily coriaceous, yellowish brown usually flushed with red or purple; plants heavily pruinose, particularly the lower surface of the leaves ..... 1. C. LUTEA
    cc. Spikes relatively broad, the bracts widely spreading or nearly horizontal to the peduncle; plants not pruinose.
      d. Bracts glabrous or essentially so, chartaceous, rich yellow, nearly horizontal to the peduncle, not recurved at the tip or scarcely so; leaves ovate to broadly elliptic ..... 2. C. INSIGNIS
      dd. Bracts densely pilose, membranaceous, pale yellowish green, widely spreading, rather strongly recurved at the tip; leaves oblong to oblong-lanceolate ..... 3. C. LASIOSTACHYA
  bb. Spikes borne upon naked scapes; bracts very few, relatively distant, not imbricated at anthesis, densely pilose ..... 4. C. VILLOSA
aa. Bracts spiral.
  b. Spikes borne upon leafy stems.
    c. Stem leaves distant, occasionally approximate in lax groups of 2–3.
      d. Bracts green, rarely yellow, relatively numerous (about 15–30); petioles of upper stem leaves usually fairly elongate and only partly callous.
        e. Spikes with relatively long peduncles; bracts green, broadly obtuse or rounded, frequently lacerate in age.
          f. Bracts deep green, closely imbricated at anthesis or only slightly spreading at the tip; flowers sharply ascending, deep violet or pale yellow; plants 1.0–2.0 m. tall ..... 5. C. ALLOUIA
          ff. Bracts pale green, irregularly reflexed or spreading at anthesis; flowers lax, pale yellow; plants 2.0–2.5 m. tall ..... 6. C. INDECORA
        ee. Spikes sessile or with a much shorter peduncle; bracts yellow, deeply round-emarginate, usually with an inconspicuous central cusp, closely imbricated at anthesis; flowers yellow; plants about 1 m. tall ..... 7. C. ALLENII
      dd. Bracts white, yellow, or bronze, relatively few (about 5–15); petioles of upper stem leaves usually very short and wholly callous; flowers white.
        e. Bracts yellow, about 10–15, imbricated at anthesis, tomentose toward the base, chartaceous; peduncles glabrous; petioles of upper stem leaves 1–3 cm. long, the sheaths obtuse, not auriculate ..... 8. C. LAGUNAE

ee. Bracts bronze, 5–10, widely spreading, glabrous, chartaceous; peduncles glabrous; petioles of upper stem leaves 0.3–0.6 cm. long, the sheaths auriculate ..... 9. C. PICTA

eee. Bracts white or yellowish at the base, about 5–10, loosely sheathing, glabrous, delicately membranaceous; peduncles densely and minutely puberulent; petioles of upper stem leaves 0.5–2.0 cm. long ..... 10. C. WARSCEWICZII

cc. Stem leaves densely whorled beneath the inflorescence; bracts laxly spreading at anthesis, tomentose ..... 11. C. FOLIOSA

bb. Spikes borne upon leafless scapes.

c. Spikes subsessile or very shortly (about 1–3 cm.) pedunculate, the bracts relatively few, 3–4 cm. long, membranaceous, green or tinged with red; flowers bright yellow, 2.5–4.0 cm. long; plants 3–4 dm. tall ..... 12. C. PANAMENSIS

cc. Spikes with elongate peduncles.

d. Plants massive, 1.5–5.0 m. tall; spikes broadly ovoid, 5–12 cm. long, very dense; bracts numerous, subcoriaceous, yellow flushed with red, irregularly lacerate; flowers pale greenish yellow, 3.0–3.5 cm. long ..... 13. C. ALTISSIMA

dd. Plants slender, 1.5–4.0 dm. tall; spikes turbinate, 1.5–2.5 cm. long, rather lax; bracts few, foliaceous, lanceolate or ovate-lanceolate, entire; flowers white, 1.0–1.5 cm. long ..... 14. C. MICROCEPHALA

Fig. 45. *Calathea lutea*

1. CALATHEA LUTEA (Aubl.) G. F. W. Meyer, Prim. Fl. Esseq. 10. 1818.

Maranta lutea Aubl. Hist. Pl. Guian. 1:4. 1775.
Maranta Casupo Jacq. Fragm. Bot. 51. *pl. 63, fig. 4.* 1809.
Maranta Cachibou Jacq. loc. cit. 52. *pl. 69-70.* 1809.
Calathea discolor G. F. W. Meyer, loc. cit. 7. 1818.
Calathea Casupito G.F.W. Meyer, loc. cit. 10. 1818.
Calathea marantina K. Koch. in Gartenzeit. 25:163. 1857.

Plants very stout, 1–5 m. tall, caulescent; leaves long-petiolate, broadly elliptic to suborbicular, obtuse or abruptly acuminate, the base obtuse or rounded then very abruptly and shortly decurrent, 20–150 cm. long, 15–60 cm. broad, glabrous, conspicuously pruinose, particularly the lower surface; callus 5–12 cm. long; inflorescence terminating the leafy stem, consisting of 2–several pedunculate, flattened, oblong, ellipsoid spikes 15–40

cm. long, 3–5 cm. broad; bracts 2-ranked, more or less conduplicate, nearly orbicular, 3.5–4.5 cm. long and broad, sharply ascending and nearly parallel to the rachis, heavily coriaceous, yellowish brown usually flushed with red or purple, glabrous to rather conspicuously appressed-tomentose; flowers white or very pale yellow, occasionally purplish, 4.0–4.5 cm. long, well exserted from the bracts.

British Honduras to Brazil and Peru; Antilles, in coastal thickets and marshes.

BOCAS DEL TORO: Isla Colón, *von Wedel 311;* Old Bank Island, *von Wedel 1996.* CANAL ZONE: Río Indio, *Dodge & Allen 17474;* Cerro Gordo, *Standley 26016;* Barro Colorado Island, *Standley 40835;* Gatún, *Hayes 820.* COCLÉ: Penonomé, *Williams 387.* COLÓN: Catival, *Standley 30228.* CHIRIQUÍ: San Bartolomé, *Woodson & Schery 914.* DARIÉN: Boca de Cupe, *Allen 880;* Río Sambú, *Pittier 5559.* PANAMÁ: Río Tecúmen, *Standley 29453.*

One of the most common and striking plants of coastal marshes. The lower surface of the leaves is covered with wax which shreds off as thin flakes. Popularly but ambiguously known as *Platanillo*, and, more appropriately, *Hoja blanca.*

2. CALATHEA INSIGNIS Peters. in Mart. Fl. Bras. 3^3:124. 1890.

Calathea quadratispica Woodson in Ann. Missouri Bot. Gard. 26:278. 1939.

Plants stout, caulescent, 2–3 m. tall; leaves long-petiolate, ovate to ovate-elliptic, shortly acuminate, broadly rounded or obtuse at the base, 35–75 cm. broad, glabrous; callus stout, 4–15 cm. long; inflorescence terminating the leafy stem, consisting of 1–3 pedunculate, flattened, broadly oblong spikes 15–40 cm. long, 3–6 cm. broad; bracts 2-ranked, conduplicate, broadly reniform, 3.0–3.5 cm. long, 5–6 cm. broad, nearly horizontal to the rachis, heavily chartaceous, bright yellow, glabrous or very indefinitely puberulent-papillate; flowers 3.0–3.5 cm. long, pale yellow.

Mexico to Colombia and Peru, in coastal thickets and marshes.

BOCAS DEL TORO: Río Cricamola, *Woodson, Allen & Seibert 1913;* Isla Colón, *von Wedel 312.* CANAL ZONE: Frijoles, *Pittier 2684;* Barro Colorado Island, *Standley 41003.* CHIRIQUÍ: Río Gariché, *Seibert 363.* COLÓN: Río Sirrí, *Pittier 4012;* Porto Bello, *Pittier 2486.*

3. CALATHEA LASIOSTACHYA Donn. Sm. in Bot. Gaz. 31:124. 1901.

Plants fairly stout, about 2 m. tall, caulescent; leaves long-petiolate, narrowly oblong to oblong-lanceolate, shortly acuminate, the base broadly obtuse, 40–75 cm. long, 10–18 cm. broad, glabrous; callus 4–6 cm. long; inflorescence terminating the leafy stem, consisting usually of 2 long-pedunculate spikes 9–14 cm. long, 5–7 cm. broad; bracts 2-ranked, strongly conduplicate, broadly oblong-elliptic, rounded above and below, 3.5–4.0 cm. long, 2.0–2.5 cm. broad, widely spreading, rather strongly recurved at the tip, membranaceous, pale yellowish green, densely pilose; flowers 3–4 cm. long, pink or cream tinged with pink.

Costa Rica and Panama, chiefly in wet foothill thickets and open forest.

CANAL ZONE: Caño Quebrada, *Pittier 6826.* COCLÉ: El Valle de Antón, *Woodson & Schery 197;* La Mesa, *Allen 2734.* COLÓN: Fato, *Pittier 4103.*

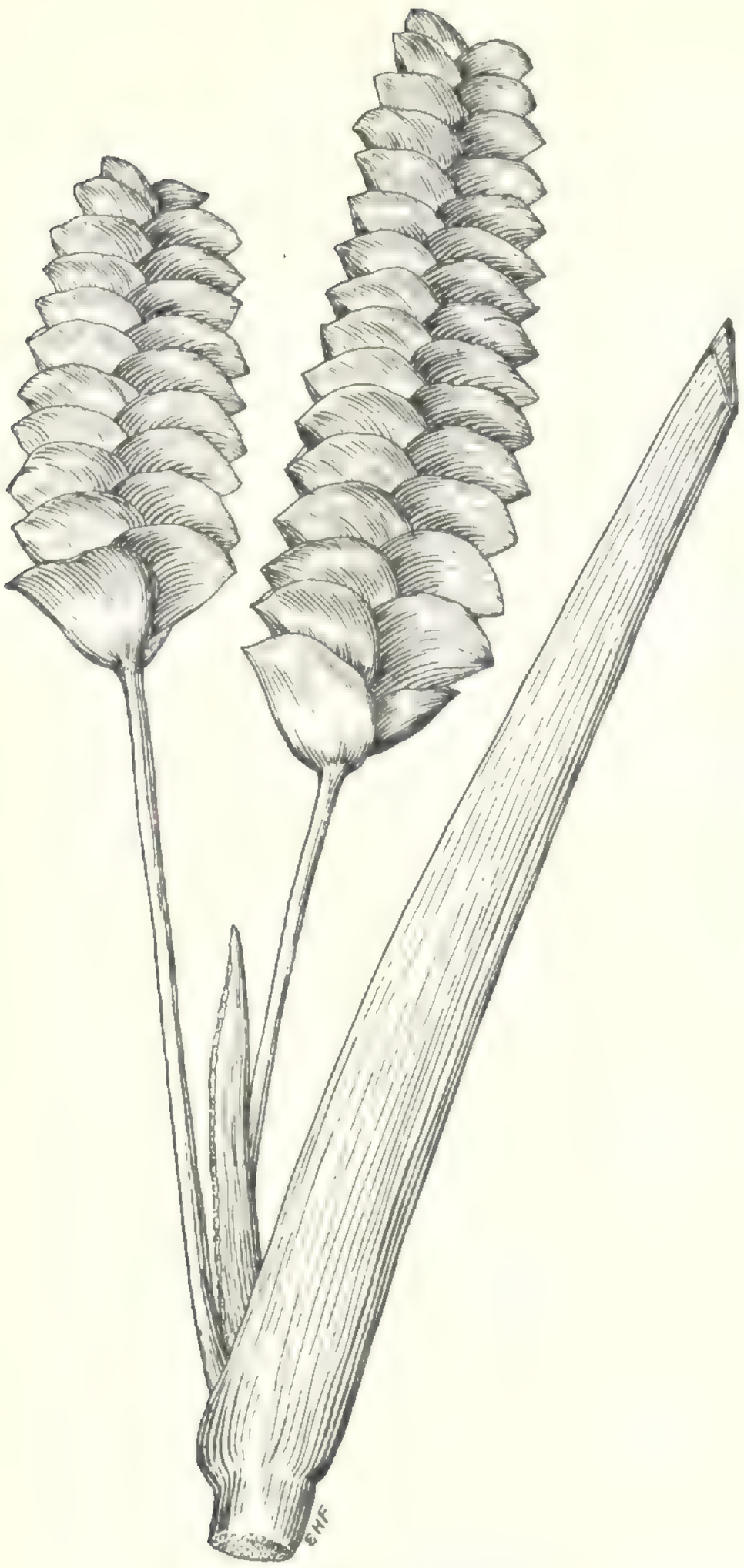

Fig. 46. *Calathea insignis*

Fig. 47. *Calathea lasiostachya*

4. CALATHEA VILLOSA (Lodd.) Lindl. in Bot. Reg. *pl. 14.* 1845.

Phrynium villosum Lodd. in Sweet, Hort. Brit. 392. 1826.
Calathea pardina Planch. & Lind. Prix Courant Fl. Nouv. 2. 1855; Fl. Serres 11:55. *pl. 1101, 1102.* 1856.
Calathea hirsuta Standl. in Jour. Wash. Acad. Sci. 15:4. 1925.

Plants of moderate size, 5–8 dm. tall, acaulescent; leaves all basal, oblong-elliptic, shortly acuminate, base obtuse to broadly acute, 15–60 cm. long, 8–15 cm. broad, scatteringly pilosulose beneath, particularly the midrib; petiole 1.5–6.0 cm. long, pilose, callous throughout or for about half its length; sheaths narrow, 30–45 cm. long, pilose; inflorescence terminating a slender leafless pilose scape

Fig. 48. *Calathea villosa*

40–75 cm. long; bracts 4–7, 2-ranked, somewhat compressed, not imbricated, rather distant, broadly ovate, shortly acuminate, 2–3 cm. long, 1.5–2.0 cm. broad, foliaceous, densely pilose; flowers 3.5–4.0 cm. long, yellow.

Costa Rica to Brazil, chiefly in lowland thickets and open forests.

CANAL ZONE: Las Cruces, *Seibert 581;* Cerro Cabra, *Allen 2021;* Ancón Hill, *Standley 25163.* PANAMÁ: Bejuco, *Woodson, Allen & Seibert 1681.*

The leaves of this species usually are mottled with splotches of deep blue-green.

5. CALATHEA ALLOUIA (Aubl.) Lindl. in Bot. Reg. *pl. 1210.* 1827; Woodson & Schery in Ann. Missouri Bot. Gard. 29:332. 1942.

Maranta Allouia Aubl. Hist. Pl. Guian. 1:3. 1775.
Curcuma americana Lam. Encycl. Meth. Bot. 2:228. 1806.

Calathea grandifolia Lindl. in Bot. Reg. *pl. 1210.* 1827.
Phrynium cylindricum Rosc. Monandr. Pl. *pl.* [*40*]. 1828.
Calathea cylindrica (Rosc.) K. Sch. in Engl. Pflanzenreich. IV. 48:83. 1902.
Calathea macrosepala K. Sch. loc. cit. 84. 1902.

Plants rather stout, caulescent, 1–2 m. tall; leaves oblong to oblong-elliptic, obtuse to very shortly acuminate, base broadly obtuse or rounded, 15–45 cm. long, 8–25 cm. broad, glabrous; petiole 2–20 cm. long, wholly callous or only in the upper portion; sheath 15–30 cm. long, inconspicuously pilosulose; spikes terminating the leafy stem, solitary; peduncle 7–20 cm. long, pilosulose; bracts about 15–30, closely imbricated except at the spreading canaliculate tip, ovate-subreniform, broadly obtuse or rounded, 1.5–2.0 cm. long, 2–3 cm. broad, foliaceous, pilosulose; flowers 3–4 cm. long, sharply ascending, pale yellow or violet.

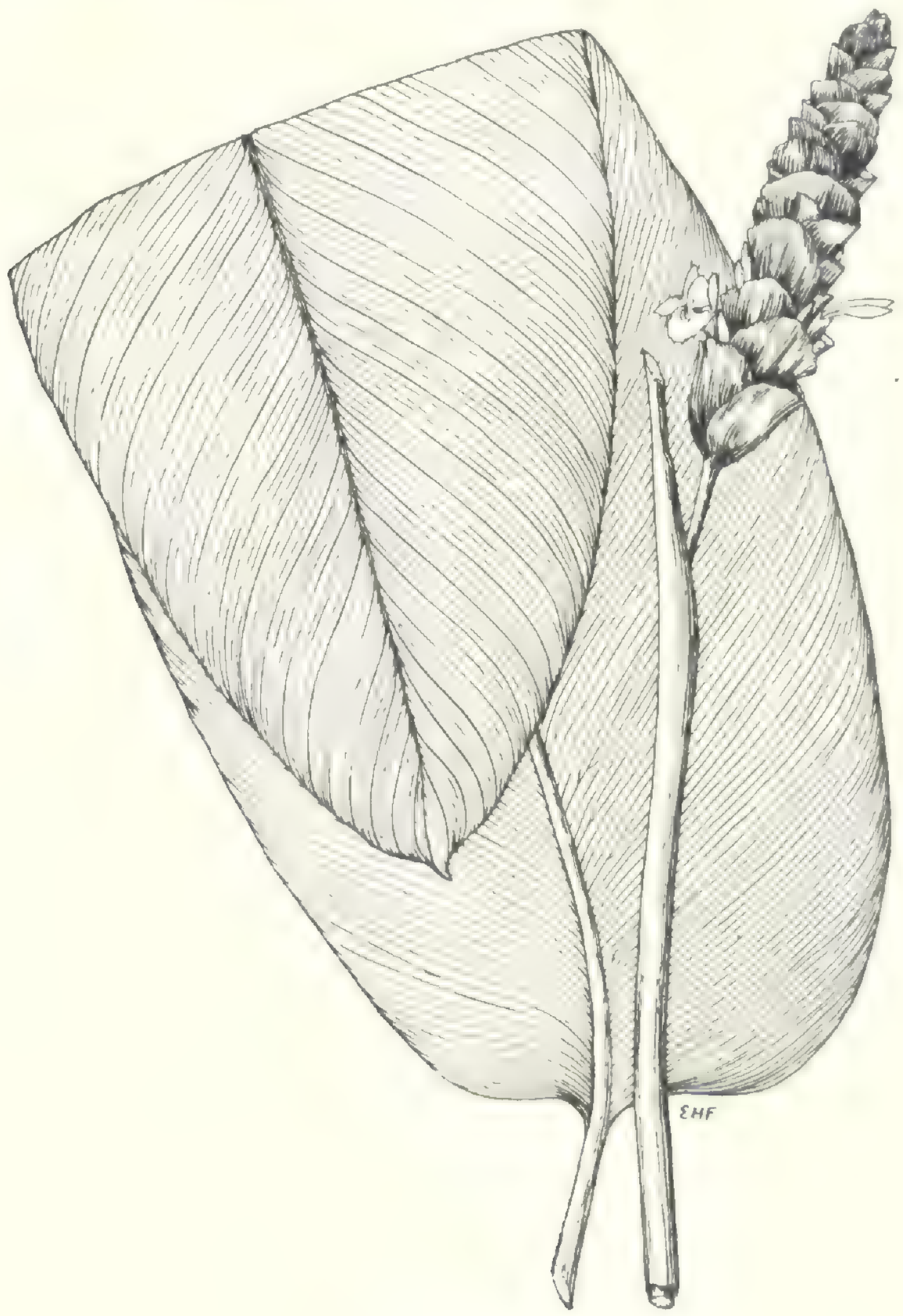

Fig. 49. *Calathea Allouia*

British Honduras to Brazil and Peru, chiefly in lowland thickets and open forest.

CANAL ZONE: Frijoles, *Killip 3430;* Gatún, *Pittier 6746;* Barro Colorado Island, *Shattuck 487.* CHIRIQUÍ: San Felix, *Pittier 5150.* COCLÉ: Cerro Valle Chiquito, *Seibert 647.* COLÓN: Palenque, *Pittier 4129.* DARIÉN: Yape, *Allen 861.* PANAMÁ: Bella Vista,

Killip 12032; Sabanas, *Paul 103;* Río Tataré, *Woodson & Schery 1011* (yellow-flowered), *Woodson & Schery 1010* (violet-flowered); between Pacora and Chepo, *Woodson, Allen & Seibert 1669* (yellow-flowered), *Woodson, Allen & Seibert 1670* (purple-flowered).

This species is common, particularly in the savannas east of Panama City, and produces yellow or violet-colored flowers in about equal numbers. The two color forms are distinguishable by no morphological characters; hence both are cited in the preceding list of exsiccatae, as collectors' labels frequently neglect notes on flower color. The yellow form is typical of the species as originally described; the violet color form may be distinguished as follows:

Fig. 50. *Calathea indecora*

5a. CALATHEA ALLOUIA (Aubl.) Lindl. var. VIOLACEA (Rosc.) Woodson in Ann. Missouri Bot. Gard. 29:332. 1942.

Phrynium violaceum Rosc. Monandr. Pl. *pl.* [*37*]. 1828.
Maranta clavata Velloso, Fl. Flum. 1: *pl. 9*. 1828.
Phrynium floribundum Lem. Jard. Fleur. 2: misc. 96. *pl. 189*. 1852.

6. CALATHEA INDECORA Woodson in Ann. Missouri Bot. Gard. 29:333. 1942.

Stout plants 2.0–2.5 m. tall, caulescent; leaves long-petiolate, oblong-elliptic, shortly acuminate, base rounded, 40–65 cm. long, 14–22 cm. broad, minutely puberulent beneath, midrib puberulent above, otherwise glabrous; petiole 20–45 cm. long, minutely pilosulose, the callus about 4 cm. long, densely papillate; sheath 11–20 cm. long, not auriculate; spikes broadly ovoid, 5–8 cm. long; peduncle stout, 10–18 cm. long, puberulent above; bracts about 15–30, very broadly ovate, about 2 cm. long and broad, pale green, densely pilosulose, lacerate and loosely spreading at anthesis; flowers loosely spreading at anthesis, 2.5–3.0 cm. long, pale yellow.

Panama, in lowland thickets.

BOCAS DEL TORO: Isla Colón, *von Wedel 476;* Old Bank Island, *von Wedel 2102;* Water Valley, *von Wedel 712.*

Closely related to *C. Allouia,* but easily distinguished in the field by its larger size and disorderly appearance of the spikes. Probably occurs in adjacent Costa Rica.

7. CALATHEA ALLENII Woodson in Ann. Missouri Bot. Gard. 29:331. 1942.

Plants rather stout, caulescent, about 1 m. tall; leaves fairly long-petiolate, oblong-elliptic, abruptly subcaudate-acuminate, base rounded, 20–45 cm. long, 8–15 cm. broad, glabrous except the midrib puberulent beneath; petiole 20–25 cm. long, pilosulose, callus about 5 cm. long; sheath 7–10 cm. long, 4 cm. broad, pilosulose without; spikes broadly fusiform, 11–13 cm. long, 3.0–3.5 cm. broad, sessile or with a densely pilosulose peduncle 4 cm. long; bracts spiral, about 20–25, densely imbricated, oblong or the lower broadly oval, round-emarginate with a minute median cusp, 5.0–5.5 cm. long, 1.5–3.5 cm. broad, yellow, the margins and tip pilosulose to glabrate; flowers ascending, 3.5–4.0 cm. long, yellow.

Panama, in highland forest.

PANAMÁ: Cerro Campana, *Allen 2218.*

8. CALATHEA LAGUNAE Woodson in Ann. Missouri Bot. Gard. 29:333. 1942.

Plants nearly 1 m. tall, caulescent; leaves very shortly petiolate, oblong-ellpitic or oval, 11–45 cm. long, 7–12 cm. broad, glabrous; petiole 1–6 cm. long, callous throughout and minutely papillate; sheath 6–15 cm. long, obtuse, glabrous; spikes ovoid, 3–6 cm. long, peduncle 10–17 cm. long, pilosulose above, otherwise glabrous; bracts spiral, about 10–15, imbricated, subreniform-ovate, broadly obtuse

or rounded, 1–2 cm. long, yellow, densely pilosulose particularly at the base; flowers ascending, 2.0–2.5 cm. long, white.

Panama, in lowland thickets.

BOCAS DEL TORO: Western River, *von Wedel 2706;* Isla Colón, *von Wedel 1328.*

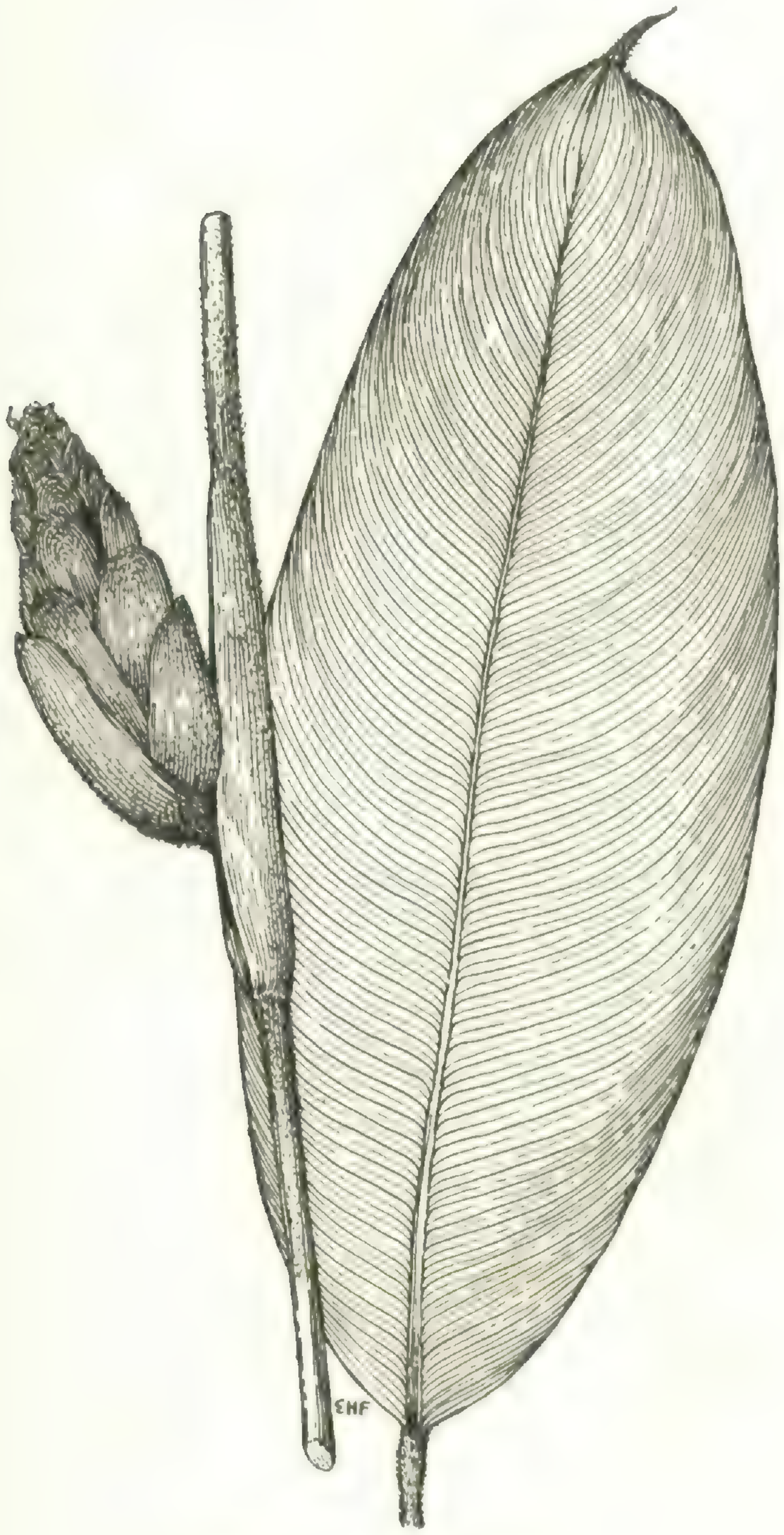

Fig. 51. *Calathea Allenii*

Fig. 52. *Calathea lagunae*

9. CALATHEA PICTA Hook. f. in Bot. Mag. *pl. 7674.* 1899; Woodson & Schery in Ann. Missouri Bot. Gard. 29:334. 1942.

Plants 1.0–1.5 m. tall, caulescent; leaves broadly oblong-elliptic, very shortly and abruptly acuminate, base rounded-subtruncate, 15–30 cm. long, 7–14 cm. broad, glabrous except the puberulent midrib above; petiole 0.3–1.0 cm. long, wholly callous; sheaths 10–15 cm. long, auriculate at the petiole, glabrous; spikes ellipsoid, 4–6 cm. long; peduncle 13–20 cm. long, glabrous; bracts about 5–10,

Fig. 53. *Calathea picta*

widely spreading, chartaceous, glabrous, bronze-colored, broadly ovate-subreniform, 1.5–2.0 cm. long, 2.0–2.5 cm. broad, shortly apiculate; flowers 5.0–5.5 cm. long, widely exserted, white.

Panama; originally published from cultivated plants presumably obtained from Brazil. In Panama occurring in foothill and highland forest and thickets.

COCLÉ: El Valle de Antón, *Allen 1217;* La Mesa, *Allen 2331.* PANAMÁ: Cerro Campana, *Allen 2219.*

10. CALATHEA WARSCEWICZII (Mathieu) Koernicke in Gartenfl. 7:87. 1858.

Maranta Warscewiczii Mathieu ex Planch. in Fl. Serres 9:209. *pl. 939-940.* 1854.
Phrynium Warscewiczii (Mathieu) Klotzsch in Allgem. Gartenzeit. 23:89. 1855.

Plants rather slender, caulescent, 0.5–1.0 m. tall; leaves oblong-oval, abruptly and shortly acuminate or subcaudate-acuminate, base obtuse or rounded, 9–30 cm. long, 5–10 cm. broad, minutely puberulent to glabrous, the lower surface purple, the upper variegated with white or light green; petiole 0.5–2.0 cm. long, wholly callous; sheaths 9–15 cm. long, obtuse, minutely puberulent; spikes ellipsoid-turbinate, 3.5–6.0 cm. long; peduncle 5–12 cm. long, minutely pilosulose; bracts about 5–10, loosely sheathing, obovate, shortly apiculate, 2–4 cm. long, 3–5 cm. broad, membranaceous, white or yellowish at the base, glabrous; flowers 3.5–4.0 cm. long, widely exserted, white.

Costa Rica and Panama, in moist lowland and foothill forests.

BOCAS DEL TORO: Water Valley, *von Wedel 1586, 2655;* Isla Colón, *von Wedel 1270;* Rubbertree Station, vicinity of Almirante, *Stork 119.*

A very lovely species sometimes found in northern greenhouses.

11. CALATHEA FOLIOSA Rowlee ex Woodson in Ann. Missouri Bot. Gard. 29:332. 1942.

Plants rather slender, caulescent, about 1 m. tall or less; stem leaves densely whorled beneath the flowering peduncle, oblong-oblanceolate, shortly acuminate, base obtuse, 25–35 cm. long, 7–8 cm. broad, glabrous except the puberulent mid-rib beneath; petiole about 1 cm. long, wholly callous; sheaths 15–20 cm. long, obtuse, glabrous; spikes subglobose, about 6 cm. long; peduncle about 9 cm. long, glabrous; bracts about 25, more or less imbricated, broadly ovate, shortly acuminate, 2–3 cm. long, densely tomentellous without; flowers about 3.0–3.5 cm. long, yellowish.

Panama, probably also in adjacent Costa Rica, in lowland thickets.

BOCAS DEL TORO: Farm No. 6, near Almirante, *Blair 1016.*

Noteworthy for the whorled stem leaves. Possibly a form of *C. indecora.*

12. CALATHEA PANAMENSIS Rowlee, ex Standl. Jour. Wash. Acad. Sci. 15:4. 1925.

Plants of mediocre size, acaulescent; leaves oblong- or obovate-elliptic, very abruptly and shortly acuminate to obtuse or rounded, base very broadly obtuse or rounded, 15–20 cm. long, 7–10 cm. broad, glabrous; petioles 0.5–1.0 cm. long, wholly callous, minutely puberulent; sheaths 8–16 cm. long, narrow, obscurely

Fig. 54. *Calathea panamensis*

auriculate, glabrous or essentially so; spikes arising directly from the rhizome, subsessile or very shortly (about 4 cm.) pedunculate; bracts about 5–10, ovate-lanceolate, acute to acuminate, 3–4 cm. long, membranaceous, green or tinged with red, sparsely pilosulose to glabrate; flowers 2.5–4.0 cm. long, bright yellow. Costa Rica and Panama, in lowland thickets and savannas.

PANAMÁ: Cabuya, *Allen 2556;* Río Tataré, *Woodson & Schery 1007;* Río Tapia, *Maxon & Harvey 6664;* between Pacora and Chepo, *Woodson, Allen & Seibert 1644;* Matías Hernández, *Pittier 6806.*

These plants may eventually be found to be conspecific with one of the South American species of the *C. chrysoleuca* complex.

13. CALATHEA ALTISSIMA (Poeppig & Endl.) Koern. in Bull. Soc. Nat. Moscow 35[1]:141. 1862.

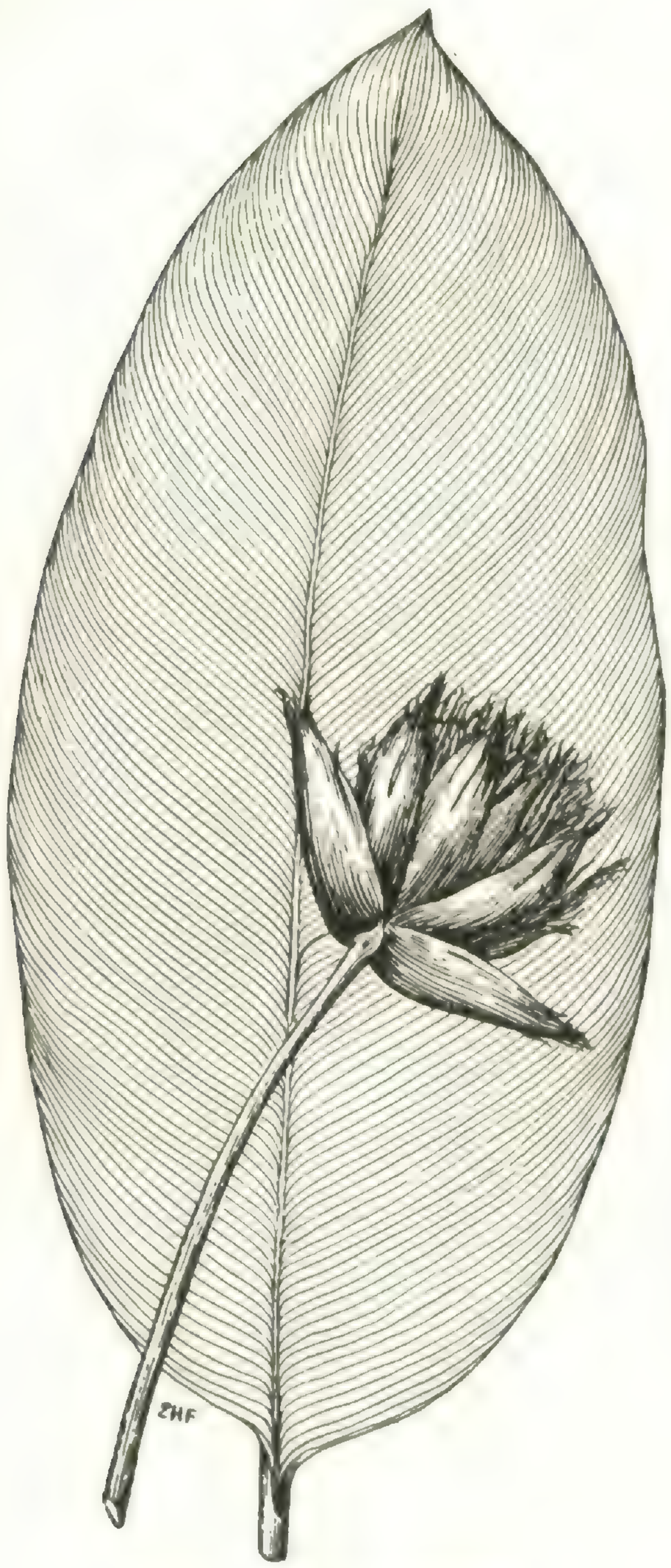

Fig. 55. *Calathea altissima*

Phrynium altissimum Poeppig & Endl. Nov. Gen. & Sp. 2:20. 1838.
Phyllodes inocephalum O. Ktze. Rev. Gen. 2:694. 1891.

Very stout acaulescent plants; leaves very long-petiolate, broadly oval, 45–70 cm. long, 25–35 cm. broad, obtuse or rounded at the tip, base rounded, glabrous; petiole stout, 9–12 dm. long, callus 4–12 cm. long, glabrous; sheath 4–8 dm. long, obtuse; spikes subglobose, very long-pedunculate, 5–12 cm. long; peduncle 5–8 dm. long, glabrous; bracts very numerous, about 5 cm. long, coriaceous, deeply and indefinitely lacerate, pale yellow flushed with rose, margins green; flowers 4–5 cm. long, pale yellow.

Guatemala to the Guianas and Peru, in lowland thickets and open forest.

BOCAS DEL TORO: Isla Colón, *von Wedel 355*. CANAL ZONE: Río Culebra, *Pittier 4159;* Matachín, *Kuntze 1916;* Barro Colorado Island, *Bailey & Bailey 16*. CHIRIQUÍ: Puerto Armuelles, *Woodson & Schery 850;* San Bartolomé, *Woodson & Schery 893*. DARIÉN: El Real, *Allen 950*.

Very conspicuous plants, the spikes looking like gigantic, over-used bottle brushes.

14. CALATHEA MICROCEPHALA (Poeppig & Endl.) Koern. in Bull. Soc. Nat. Moscow 35[1]:125. 1862; Woodson & Schery in Ann. Missouri Bot. Gard. 29:334. 1942.

Phrynium microcephalum Poeppig & Endl. Nov. Gen. & Sp. 2:20. *pl. 128, figs. a-b.* 1838.
Maranta micans Mathieu, Cat. 1853.
Calathea micans (Mathieu) Koern. loc. cit. 126. 1862.
Calathea albicans Brongn. ex K. Sch. in Engl. Pflanzenreich IV. 48:112. 1902.

Fig. 56. *Calathea microcephala*

Slender acaulescent herbs; leaves ovate- to oblong-elliptic, acute to shortly acuminate, base obtuse or rounded, 6–15 cm. long, 2.5–8 cm. broad, glabrous, lower surface usually purple, the upper variegated with white or pale green; petiole 1.5–10.0 cm. long, shortly callous above; sheaths very slender, 3–6 cm. long; spikes very long-pedunculate, turbinate, 1.5–2.5 cm. long, rather lax, the bracts few, lanceolate or ovate-lanceolate, acute to acuminate, entire, 0.7–1.3 cm. long, foliaceous, glabrous or minutely pilosulose, rather loosely sheathing; flowers white, or the labellum blue, 1.0–1.5 cm. long.

Guatemala to Brazil and Peru, in moist lowland and foothill thickets and open forest.

BOCAS DEL TORO: Isla Colón, *von Wedel 257;* Water Valley, *von Wedel 1585;* Old Bank Island, *von Wedel 1919;* Nievecita, *Woodson, Allen & Seibert 1815.* CANAL ZONE: Frijoles, *Standley 27534;* between Gorgona and Gatún, *Pittier 2277.* COCLÉ: El Valle de Antón, *Woodson & Schery 186, Allen 2174.*

A lovely species frequently found in northern greenhouses.

2. MYROSMA L. f.

MYROSMA L. f. Suppl. 8, 80. 1781.

Saranthe Eichl. in Abh. Akad. Berlin 1883:85. 1883, in part.
Ctenanthe Eichl. loc. cit. 83. 1883, in part.

Rhizomatous, perennial, subacaulescent herbs; leaves mostly basal, the flowering stem bearing relatively few, 2-ranked; inflorescence racemose, subspiciform, simple or infrequently branched, solitary or clustered; bracts persistent, usually relatively distant and not closely imbricated at anthesis, membranaceous or chartaceous, foliaceous or yellow, subtending clusters of rather small and inconspicuous bracteolate flowers; flowers perfect, very asymmetric, epigynous; sepals 3, more or less connate at the base, persistent, the lobes essentially equal; corolla lobes 3, unequal, shortly connate at the base; fertile stamen 1, petaloid, united at the base with 4 more or less petaloid staminodia, the outer 2 exceeding the corolla lobes; ovary inferior, 1-celled; fruit a loculicidally dehiscent 1-seeded capsule.

a. Bracts green; flowers distinctly pedicellate, conspicuously exserted from the bracts ..... 1. M. PANAMENSIS
aa. Bracts deep yellow; flowers sessile or subsessile, included within the bracts ..... 2. M. DASYCARPA

1. MYROSMA PANAMENSIS Standl. in Jour. Wash. Acad. Sci. 15:4. 1925.

Plants fairly stout, caulescent, 3–6 dm. tall; leaves obovate-elliptic, obtuse to broadly acute, base obtusely cuneate, 15–40 cm. long, 7–16 cm. broad, inconspicuously puberulent toward the base above, otherwise glabrous; petioles 0.3–1.0 cm. long, wholly callous, minutely puberulent; sheath 10–15 cm. long, glabrous; flowering stem 15–30 cm. long, bearing 2–3 somewhat reduced leaves; spikes solitary or in clusters of 2–3 at the tip of the stem, 7–12 cm. long, shortly pedunculate; bracts rather distantly 2-ranked, 14–20, foliaceous, conduplicate, obovate-oblong, 2.0–2.5 cm. long, about 1 cm. broad, broadly obtuse, nearly horizontal; flowers distinctly (about 0.3 cm.) pedicellate, exserted from the bracts, 2.0–2.5 cm. long, creamy white.

Panama, in moist lowland forest.

CANAL ZONE: Madden Dam, *Dodge, Steyermark & Allen 16502.* PANAMÁ: Río Maestra, *Allen 4;* Río Tecúmen, *Standley 26738.*

I have not been able to examine specimens of *M. Hoffmannii* K. Sch., of Costa Rica, to which this species must be very closely related.

2. MYROSMA DASYCARPA (Donn. Sm.) Woodson in Ann. Missouri Bot. Gard. 29:335. 1942.

Calathea dasycarpa Donn. Sm. in Bot. Gaz. 31:123. 1901.
Ctenanthe dasycarpa (Donn. Sm.) K. Sch. in Engl. Pflanzenreich IV. 48:153. 1902.

Stout caulescent herbs 2–4 m. tall; leaves chiefly basal, oblong-elliptic, 20–50 cm. long, 10–20 cm. broad, very shortly and abruptly acuminate, base broadly and inequilaterally obtuse or rounded, the midrib above and lower margin puberulent-ciliate, otherwise glabrous; petiole 9–20 cm. long, much shorter on the upper stem leaves, callous near the blade, matted-pilosulose; sheaths 15–25 cm. long, pilose; flowering stem 1–2 m. tall, pilose, bearing several more or less reduced leaves, dichotomously branched above, and bearing the spikes paired or in clusters of 3–5; spikes shortly pedunculate, flattened, 7–12 cm. long; bracts deep yellow, relatively distant at anthesis, strongly conduplicate, 1–2 cm. long, 0.8–1.0 cm. broad at the middle, broadly acute, the margins densely ciliate to glabrate; flowers 1.0–1.5 cm. long, sessile or subsessile, white.

Costa Rica and Panama, in moist foothill forest.

BOCAS DEL TORO: Isla Colón, *von Wedel 433.* COCLÉ: El Valle de Antón, *Woodson & Schery 165.* PANAMÁ: Cerro Campana, *Allen 2221.*

Fig. 57. *Myrosma dasycarpa*

3. ISCHNOSIPHON Koernicke

ISCHNOSIPHON Koern. in Nouv. Mem. Soc. Nat. Moscow 11:346. *pl. 10-11.* 1859.
Pleiostachya K. Sch. in Engl. Pflanzenreich IV. 48:164. 1902.

Stout rhizomatous, caulescent, perennial herbs; leaves 2-ranked, chiefly basal; inflorescence spicate, solitary or clustered; bracts persistent, closely imbricated, chartaceous or subcoriaceous, subtending small clusters of sessile or subsessile flowers; sepals 3, free, equal; corolla with a long slender tube, the limb 3-lobed; staminodia 2–3, the outer solitary, petalaceous; fertile stamen 1; ovary inferior, 1-celled; fruit a 1-seeded, loculicidally dehiscent capsule.

a. Spikes narrowly cylindrical, occasionally interrupted ..... 1. I. LEUCOPHAEUS
aa. Spikes strongly compressed, continuous.
  b. Bracts glabrous, pruinose.
    c. Spikes pedunculate ..... 2. I. PRUINOSUS
    cc. Spikes sessile ..... 3. I. PITTIERI
  bb. Bracts conspicuously ferruginous-pilose ..... 4. I. MORLAEI

1. ISCHNOSIPHON LEUCOPHAEUS (Poeppig & Endl.) Koernicke in Bull. Soc. Nat. Moscow 35[1]:91. 1862.

Calathea leucophaea Poeppig & Endl. Nov. Gen. & Sp. 2:21. *pl. 129.* 1838.
Calathea leucocephala D. Dietr. Synops. 1:7. 1839.

Plants 1–2 m. tall, glabrous throughout; leaves chiefly basal, broadly oval, very abruptly and shortly acuminate, base broadly rounded-subtruncate, 15–30 cm. long, 12–20 cm. broad, glabrous, pruinose beneath; petiole 2–20 cm. long,

Fig. 58. *Ischnosiphon leucophaeus*

the callus 1.5–3.0 cm. long; sheath 6–20 cm. long; spikes usually in clusters of 2–6, sessile, narrowly cylindrical, 10–15 cm. long; bracts oblong, obtuse, 2.5–3.0 cm. long, glabrous, somewhat pruinose; flowers 3–4 cm. long, white.

Panama to Brazil, in coastal swamps and lowland thickets.

CANAL ZONE: Río Chagres, *Fendler 337;* Barro Colorado Island, *Standley 41010;* Ft. Sherman, *Standley 31067.* BOCAS DEL TORO: Isla Colón, *von Wedel 252.* PANAMÁ: Taboga Island, *Standley 28028;* Río Tapia, *Standley 28126.*

2. ISCHNOSIPHON PRUINOSUS (Reg.) Peters. in Bot. Tidskr. 18:264. *pl. 18.* 1892.

Fig. 59. *Ischnosiphon pruinosus*

Maranta pruinosa Regel in Gartenfl. 27:104. 1878.
Pleiostachya pruinosa (Reg.) K. Sch. in Engl. Pflanzenreich IV. 48:165. 1902.

Plants stout, 2–3 m. tall; leaves mostly basal, long-petiolate, oblong-elliptic, abruptly and shortly acuminate, base very broadly obtuse or rounded, 20–45 cm. long, 12–18 cm. broad, glabrous; petiole 6–30 cm. long, the upper portion stoutly callous; sheath 30–45 cm. long; spikes in 2–3 long (10–20 cm.) pedunculate clusters, laterally compressed, 12–16 cm. long, about 1.5 cm. broad; bracts closely imbricated, ovate-oblong, acute, 3–5 cm. long, pale green to yellowish, glabrous, pruinose; flowers 4–5 cm. long, white, the outer staminode frequently purple.

British Honduras to Panama, in lowland thickets.

Fig. 60. *Ischnosiphon Morlaei*

CHIRIQUÍ: San Bartolomé, *Woodson & Schery 888.* CANAL ZONE: Quebrada Ancha, *Steyermark & Allen 17129;* Ft. Sherman, *Standley 31092;* Gatún, *Hayes 110;* Barro Colorado Island, *Standley 41052.* PANAMÁ: Río Tapia, *Standley 411;* Juan Díaz, *Killip 3110.*

3. ISCHNOSIPHON PITTIERI (Rowlee) Woodson in Ann. Missouri Bot. Gard. 29:335. 1942.

Pleiostachya Pittieri Rowlee ex Standl. Jour. Wash. Acad. Sci. 15:5. 1925.

Plants about 1 m. tall; leaves mostly basal, elliptic-oblong, shortly and abruptly acuminate, base rounded, 30–45 cm. long, 12–15 cm. broad, glabrous; petiole and sheath 60–75 cm. long, the callus about 4 cm. long; spikes paired, sessile, 15–18 cm. long, about 1.3 cm. broad, strongly flattened; bracts ovate, acute, 2.0–2.5 cm. long, closely imbricated, glabrous, pale yellowish green with conspicuous red margins; flowers 3.5–4.0 cm. long, white.

Panama, in lowland forest, probably also in northwestern Colombia.

SAN BLAS: Puerto Obaldía, *Pittier 4409.*

4. ISCHNOSIPHON MORLAEI Eggers in Bot. Centralbl. 53:307. 1893.

Pleiostachya Morlaei (Eggers) K. Sch. in Engl. Pflanzenreich IV. 48:165. 1902.

Plants 1.5–2.0 m. tall; leaves mostly basal, narrowly ovate-elliptic, shortly and abruptly acuminate, base broadly rounded, 40–75 cm. long, 12–25 cm. broad, essentially glabrous; petiole 15–20 cm. long, the upper portion callous; sheath 20–30 cm. long; spikes clustered at the tip of paired peduncles 9–15 cm. long, strongly compressed, 12–15 cm. long, 1.5–2.0 cm. broad; bracts oblong-ovate, acute, 4–5 cm. long, pale yellowish green, very conspicuously ferruginous-pilose; flowers 4.0–4.5 cm. long, white, the outer staminodium usually purple.

Costa Rica to Ecuador, in lowland thickets and open forest, frequently in marshes.

BOCAS DEL TORO: Isla Colón, *von Wedel 208;* lower Changuinola River, *Stork 79.* DARIÉN: El Real, *Allen 965.*

4. MARANTA L.

MARANTA L. Sp. Pl. 2. 1753.

Caulescent herbs from tuberiferous rhizomes; leaves both basal and cauline; stem branching rather diffusely and bearing at the tips rather loose racemiform cymes of rather small, pedicellate flowers subtended by caducous, somewhat foliaceous bracts; sepals 3, free, equal; corolla with a more or less prominent tube, the lobes subequal; staminodia 3–4, the outermost 2 very conspicuous and labelliform; fertile stamen 1, petaloid; ovary inferior, 1-celled; fruit nut-like and indehiscent, 1-seeded.

1. MARANTA ARUNDINACEA L. Sp. Pl. 2. 1753.

Maranta silvatica Rosc. in Trans. Linn. Soc. 8:340. 1807.
Maranta indica Tuss. Fl. Antil. 1:183. *pl. 26.* 1808.
Maranta protracta Miq. in Linnaea 18:71. 1844.

Rather weak, diffusely branching herbs 4–8 dm. tall, essentially glabrous throughout; leaves both basal and cauline, ovate to ovate-lanceolate, rather gradually acute to acuminate, base broadly obtuse, 10–20 cm. long, 3–8 cm. broad; petiole 1.0–1.5 cm. long, wholly callous; sheaths very narrow, 5–10 cm. long; inflorescence diffusely branching, the rachis rather distantly flexuous, several- to many-flowered; bracts caducous, oblong, obviously leafless sheaths, 2–4 cm.

Fig. 61. *Maranta arundinacea*

long; pedicels 0.5–1.5 cm. long; sepals ovate-lanceolate, 1.0–1.5 cm. long, persistent and accrescent in fruit; perianth (including the staminodia) about 2 cm. long, white; fruits nut-like, ellipsoid, about 1 cm. long.

Very widely distributed throughout all tropical America, possibly indigenous to Central and northern South America. Frequently encountered in thickets and waste places.

CANAL ZONE: Ancón Hill, *Woodson, Allen & Seibert 1317;* Balboa, *Standley 26486;* Miraflores, *Pittier 3964.* CHIRIQUÍ: upper Río Chiriquí Viejo, *Seibert 405.* PANAMÁ: Isla Taboga, *Woodson, Allen & Seibert 1438;* between Panamá and Chepo, *Dodge, Hunter, Steyermark & Allen 16665;* Matías Hernández, *Standley 28965.*

Arrowroot starch, used by laundresses, is obtained from the tuberous rootstock of this plant, known in some localities as *Sagú.*

5. STROMANTHE Sond.

STROMANTHE Sond. in Hamb. Gartenzeit. 5:225. 1849.

Marantopsis Koernicke in Bull. Soc. Nat. Moscow 35[1]:97. 1862.
Kerchovea Jorissenne in Belg. Hort. 32:201. 1882.

Fig. 62. *Stromanthe lutea*

Rhizomatous caulescent perennial herbs; leaves chiefly basal, 2-ranked; inflorescence racemiform or paniculate, several- to many-flowered, the rachis closely and sharply zig-zag; bracts caducous, usually orange or yellowish, subtending small groups of inconspicuous sessile or subsessile flowers; sepals 3, free and equal; corolla with a very short tube, the lobes 3, nearly equal; fertile stamen 1, somewhat petaloid; staminodia 4, rarely 2, the outer 2 petaloid, occasionally suppressed; ovary inferior, 1-celled; fruit a 1-seeded, loculicidally dehiscent capsule.

1. Stromanthe lutea (Jacq.) Eichl. in Abh. Akad. Berlin 1883:81. 1883; Woodson & Schery in Ann. Missouri Bot. Gard. 29:334. 1942.

Maranta lutea Jacq. Collect. 4:117. 1790; Icon. Pl. Rar. 2: *pl.* [*201*]. 1786-1793.
Maranta Jacquinii R. & S. Syst. 1:558. 1818.
Marantopsis lutea (Jacq.) Koern. in Bull. Soc. Nat. Moscow 35[1]:97. 1862.
Myrosma Guapilesense Donn. Sm. in Bot. Gaz. 23:251. 1897.

Fairly stout herbs 1–2 m. tall; leaves chiefly whorled at the base of the stem, long-petiolate, oblong-elliptic, very abruptly and shortly acuminate, base broadly obtuse to rounded, 20–40 cm. long, 8–12 cm. broad, glabrous or the midrib inconspicuously puberulent above; petiole 3–5 cm. long, almost wholly callous; sheath 20–30 cm. long, scatteringly pilose to glabrate; spikes rather diffusely and paniculately compounded, the rachis closely and sharply zig-zag, the internodes about 0.3 cm. long; bracts broadly elliptic-oblong, obtuse, 1.0–2.5 cm. long, orange or deep yellow, essentially glabrous, caducous immediately after anthesis; flowers about 1 cm. or somewhat less, orange or yellow, the outer staminodia lacking.

Costa Rica to Venezuela; chiefly in lowland thickets and open forest.

Canal Zone: Barro Colorado Island, *Woodson & Schery 967*; Chagres, *Fendler 442*; Quebrada López, *Allen 2125*.

6. THALIA L.

Thalia L. Sp. Pl. 1193. 1753.

Peronia de la Roche in Redouté, Liliac. 6: *pl. 342*. 1812.
Malacarya Raf. in Amer. Month. Mag. 190. 1819.
Spirostalis Raf. Fl. Tellur. 4:51. 1836.

Rhizomatous caulescent perennial herbs; leaves 2-ranked, both basal and cauline; inflorescence laxly paniculate, the rachis closely and sharply zig-zag; bracts more or less foliaceous, caducous immediately after anthesis; sepals 3, free and equal; corolla with a very short tube, the lobes 3, equal, hyaline; fertile stamen 1, somewhat petaloid; staminodia 3, the outer solitary, conspicuously petalaceous; ovary inferior, 1-celled; fruit nut-like and indehiscent, 1-seeded.

1. Thalia geniculata L. Sp. Pl. 1193. 1753.

Maranta geniculata (L.) Lam. Tabl. Encycl. Meth. Bot. 1:9. *pl. 1, fig. 2*. 1791.
Thalia erecta Vell. Fl. Flum. 1: *pl. 17*. 1827.
Maranta flexuosa Presl, Reliq. Haenk. 1:107. 1827.

Fig. 63. *Thalia geniculata*

Thalia angustifolia Wright, ex Griseb. Cat. Pl. Cub. 256. 1866, non Peters.

Thalia altissima Klotzsch in Schomb. Reise in Brit.-Guiana 3:917. 1848.

Plants rather stout, 2–4 m. tall; leaves ovate to ovate-lanceolate, 20–75 cm. long, 5–30 cm. broad, gradually and narrowly to very abruptly and shortly acuminate, base obtuse to rounded, glabrous; petiole 30–50 cm. long, the callus very short; sheath 30–100 cm. long; inflorescence diffusely paniculate, many-flowered, the rachis slender and sharply zig-zag, with internodes 0.5–1.0 cm. long; bracts oblong-lanceolate, 1.0–2.5 cm. long, foliaceous, caducous immediately after anthesis; flowers about 1.5 cm. long, the outer staminodium purple.

Florida; Mexico to Argentina; Antilles, in lowland marshes.

CANAL ZONE: Chagres, *Fendler 338*; Barro Colorado Island, *Shattuck 683*. CHIRIQUÍ: San Felix, *Pittier 5441*. DARIÉN: El Real, *Allen 958*. HERRERA: Santa Maria, *Allen 789*. PANAMÁ: between Panamá and Chepo, *Dodge, Hunter, Steyermark & Allen 16708*; Juan Díaz, *Standley 32047*; Matías Hernández, *Standley 31925*; Río Tecúmen, *Standley 26543*.

FOREWORD

On February 2 and 3, 1945, a conference on "Gene Action in Micro-organisms" was held in St. Louis under the auspices of the Missouri Botanical Garden. The papers read at that conference and a portion of the discussion are presented here in a more permanent form.

The early decades of Genetics were largely taken up with studies of higher plants and animals. Recently it has become increasingly apparent that such micro-organisms as protozoa, fungi, and bacteria are readily investigated by genetic techniques. As a matter of fact, once the preliminary difficulties are removed they may even possess certain definite advantages for physiological and bio-chemical problems. They are so small and easy to manipulate that they may be studied directly in the laboratory in various special ways which would be impossible with larger organisms, as, for instance, in a Warburg manometer. Because of these peculiar advantages, investigators in several fields have turned to the genetics of micro-organisms in the last few years. Mycologists, parasitologists, biochemists, and immunologists have found themselves studying the same fundamental problems but with a wide variety of micro-organisms. The conference called by the Missouri Botanical Garden had as its main purpose the integration of the research work now going forward in several such laboratories.

Annals
of the
Missouri Botanical Garden

Vol. 32 APRIL, 1945 No. 2

MENDELIAN AND CYTOPLASMIC INHERITANCE IN YEASTS[1]

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The standard ellipsoidal cell of *Saccharomyces cerevisiae* is diploid (Kruis and Šatava, '18; Winge, '35). Under certain conditions its diploid nucleus undergoes meiosis and four haploid spores are produced (Lindegren and Lindegren, '44a; Lindegren and Hamilton, '44). One-, two-, three-, and four-spored asci are found, showing that many accidents may occur during the reduction division (Lindegren and Lindegren, '44a). In our work we select the four-spored asci and dissect out the four spores separately. Each ascospore grown alone produces a small cluster of round haploid cells. Genetical analysis has shown that the ascospores are of two kinds, *a* and *α* (Lindegren and Lindegren, '43b, '43c, '43d; Lindegren, '44). The legitimate diploid vegetative cells are formed by the fusion of *a* and *α* gametes, and these legitimate diploids produce four viable ascospores on reduction, thus completing the cycle. The haplophase cultures, when grown alone, often produce diploid cells by the copulation of two haplophase cells of the same mating type; we call these illegitimate (*a*/*a* and *α*/*α*) diploids because they only rarely produce four viable ascospores. The round-celled haplophase cultures often become stabilized in the haploid condition and gradually become incapable of mating with other forms after being carried in culture for some time (Lindegren and Lindegren, '44b). Asporogenous yeasts such as *Torulopsis* and *Asporomyces* probably originated in this manner.

SEGREGATION AND MUTATION

Haploid yeast cells are much smaller and more variable than diploid cells, varying more both from culture to culture and within a single culture than diploid cells. These differences are also reflected in the colonies, the diploid colonies being larger and more uniform, while haploid cultures produce smaller colonies which are usually rough and generally show considerable variation. The haplophase originates by the reduction of the diplophase at spore formation, and the

[1] This work was supported by a grant from Anheuser-Busch, Inc., St. Louis, Mo.

segregation of a heterozygote produces segregants of different genotypes. Yeasts are extraordinarily heterozygous, and a great variation of colonial forms is obtained by the isolation of single ascospore cultures. The haploid segregants are usually rough-colonied; smooth-colonied diploid cells usually produce only rough-colonied haploid segregants. Apparently considerable mutation occurs in the haplophase but generally the original segregant can be distinguished from the secondary mutants when the culture is plated out. At first, the mutants are usually slow-growing and produce small round colonies but on transfer they become adapted and stabilized and their specific colonial character becomes apparent, distinguishing them from the original segregant. Therefore, there are two mechanisms producing variation in yeasts explainable on purely genetical grounds (fig. 1):

(1) *Segregation.*—Segregation of genes of a heterozygous diploid at meiosis produces four spores, each of which develops a different type of colony.

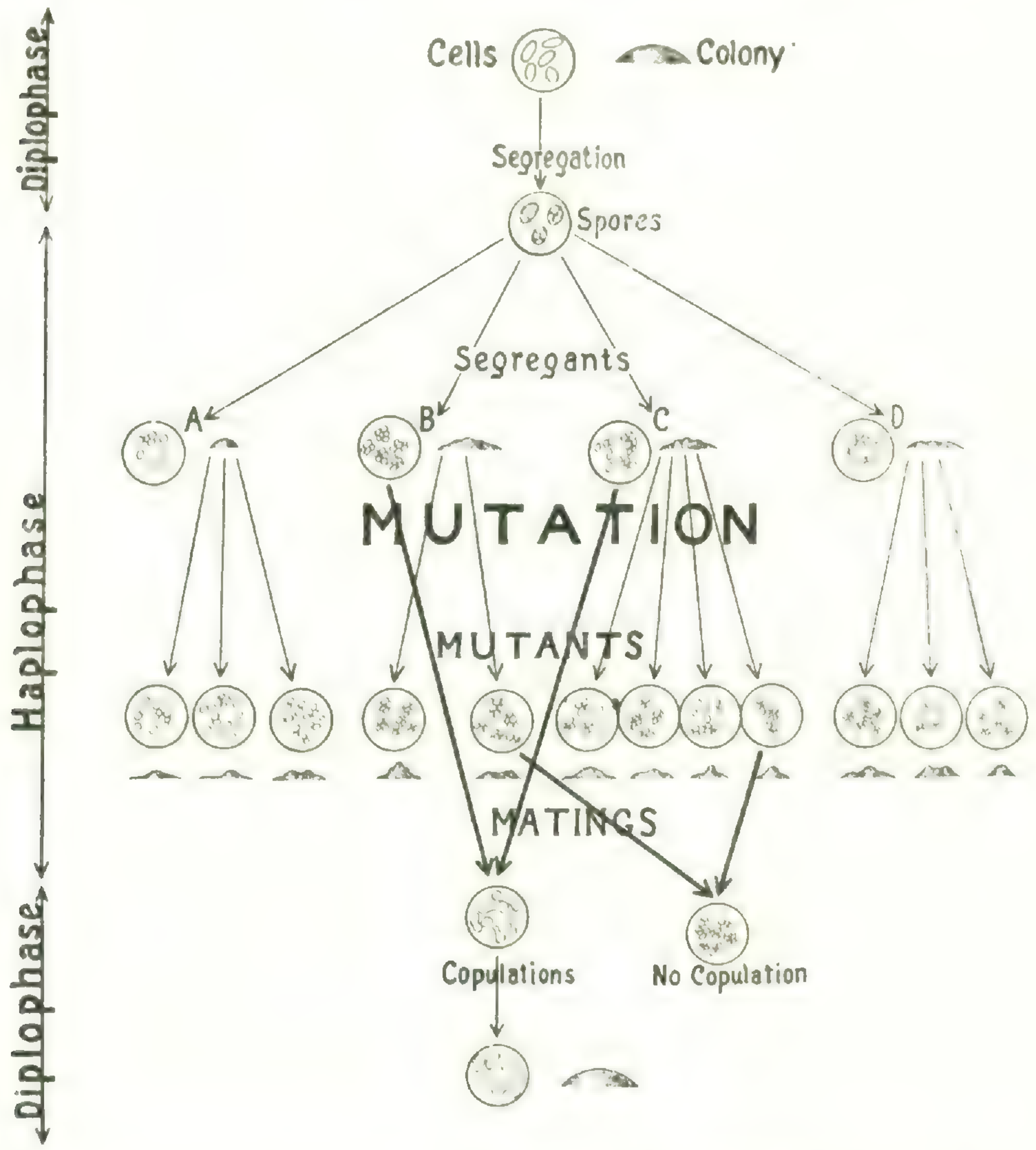

Fig. 1. Diagram showing the effects of segregation and mutation in producing variation in yeasts. The circles show the sizes and shapes of the cells as seen under the microscope and the stippled figures are profiles of the colonies on agar.

(2). *Mutation.*—Mutation in the haplophase enormously increases the variation of colonial forms. It is possible by continued subculturing to develop a tremendous variation of colonial forms from a single haplophase culture, but this usually results in loss of fertility.

DISTINCTION OF HAPLOPHASE AND DIPLOPHASE

Winge ('35) was the first to distinguish clearly between haplophase and diplophase yeast cultures, and we have corroborated his observations with some slight modifications. Workers familiar with other biological material may seriously question the propriety of speaking definitely of haplophase and diplophase in organisms where the cytological facts have not been conclusively demonstrated. I shall therefore summarize all the arguments, Winge's reinforced by ours, for distinguishing haplophase and diplophase. I should preface these rules by saying that over four-fifths of the cultures which one encounters are easily characterized by microscopic examination. They are either obviously haploid or diploid, as shown simply by size, shape, and aggregation of cells. The reasons for classifying them are as follows:

(1) The large vegetative cells which we call "legitimate diploids" produce viable four-spored asci. These spores germinate to produce smaller cells, which we call "haploid." The latter multiply vegetatively, generally maintaining their specific cell-shape and size.

(2) Two of these smaller cells may fuse to produce a large "diploid" cell capable of vegetative multiplication (Winge and Laustsen, '39a, '39b, Lindegren and Lindegren, '43b, '43d). While the large cell is undergoing vegetative reproduction, it retains its characteristic ellipsoidal shape and size. Under certain conditions, this diploid cell can be induced to sporulate. Spores from it in turn produce haploids and the process can be repeated indefinitely.

(3) The large cells which we recognize as diploids are extraordinarily stable in their genetical characteristics when they are grown under conditions in which sporulation does not occur. Transferring the cultures every forty-eight hours in broth is generally sufficient to maintain the vegetative diplophase. Colonies produced by plating out are not sectored; the plates do not show colonial variants. However, when haplophase (single ascospore) cultures of any age are plated out, a variety of colonial variants appear on the plate or the giant colonies are sectored. These facts are consistent with the view that the large cells are diploid, thus minimizing the number of spontaneous mutations found, while in the haplophase every mutant becomes apparent and is easily discovered.

(4) When the diploid cells sporulate to produce haploid cells, there is genetic evidence of a reduction division (Winge and Laustsen, '37). Genetical analysis shows that a single pair of alleles responsible for the two different mating types is segregated at this meiosis. Two *a* and two α type haplophase cultures are usually obtained from the four single ascospore cultures (Lindegren and Lindegren, '43d). There is also genetic evidence for the segregation of a gene-pair

controlling fermentation of melibiose (Lindegren, Spiegelman, and Lindegren, '44) during the meiosis that precedes spore formation. Also, evidence proving that factors controlling cell shape may be segregated in a hybrid of S. *Bayanus* and S. *cerevisiae* has been accumulated in addition to that previously offered by Winge and Laustsen ('39c) in the balanced heterozygote, *Saccharomycodes Ludwigii.*

(5) Haploid cultures of *a* and *α* mating type have been paired, and the resulting diploid cultures in turn have been induced to sporulate; the haplophases have been tested and found again to fall into the *a* and *α* categories. Matings and tests for this character have been carried through four or five generations in several cases. Similarly, segregation of the gene-pair controlling melibiose fermentation has been observed; the segregants have been tested, mated, and segregation has again been observed in the succeeding generation. Pedigrees of three or four generations are available for many characters.

The above facts seem to prove conclusively that the terms haplophase and diplophase can be used as definitely in speaking of yeasts as of organisms in which the cytological evidence is more complete. The illegitimate diploids provide an exception which is quite familiar to the mycologist. Copulations between haplophases which are usually incapable of copulating on genetical grounds were called "Durchbrechungskopulationen" by Brunswik ('24). Copulations of this type frequently occur in single ascospore cultures and produce diploids which are homozygous for the *a* or *α* factors. With rare exceptions these diploids sporulate poorly, and for this reason we have not studied them intensively. A few single ascospore cultures sporulate well, and some produce large cells that are difficult to classify either as definite haplophases or illegitimate diploids. However, the general rules laid down in the preceding discussion hold very well, and exceptions are not more frequent than one would expect on the basis of mutations, polyploidy, apomixis, or other genetical aberrations.

MATING TYPES

The variability of colonial characters is not paralleled by similar variation of mating-type specificity. With rare exceptions, each haplophase culture belongs to either the *a* or *α* mating type, or is sterile. A considerable number of sterile cultures are found and fertile cultures may become sterile, especially if the haplophase is carried a long time in culture. However, the mating types are differentiated primarily by a single pair of alleles.

A large-scale experiment has indicated that only two principal mating-type alleles are present in S. *cerevisiae*. Figure 2 shows the results of mating 58 different single ascospore cultures derived from a variety of industrial bakers' yeasts. Ly, HD, M, FLD, D, and B represent standard legitimate diploid strains of commercial baking yeasts; 800 is one of the baking strains of yeast obtained by Dr. Wickerham of the Northern Regional Research Laboratory, Peoria, Illinois. Two other cultures are hybrids, one of 800 x L and the other (800 x L) x L. Haplophases isolated from the "(800 x L) x L" hybrid were generally quite fertile.

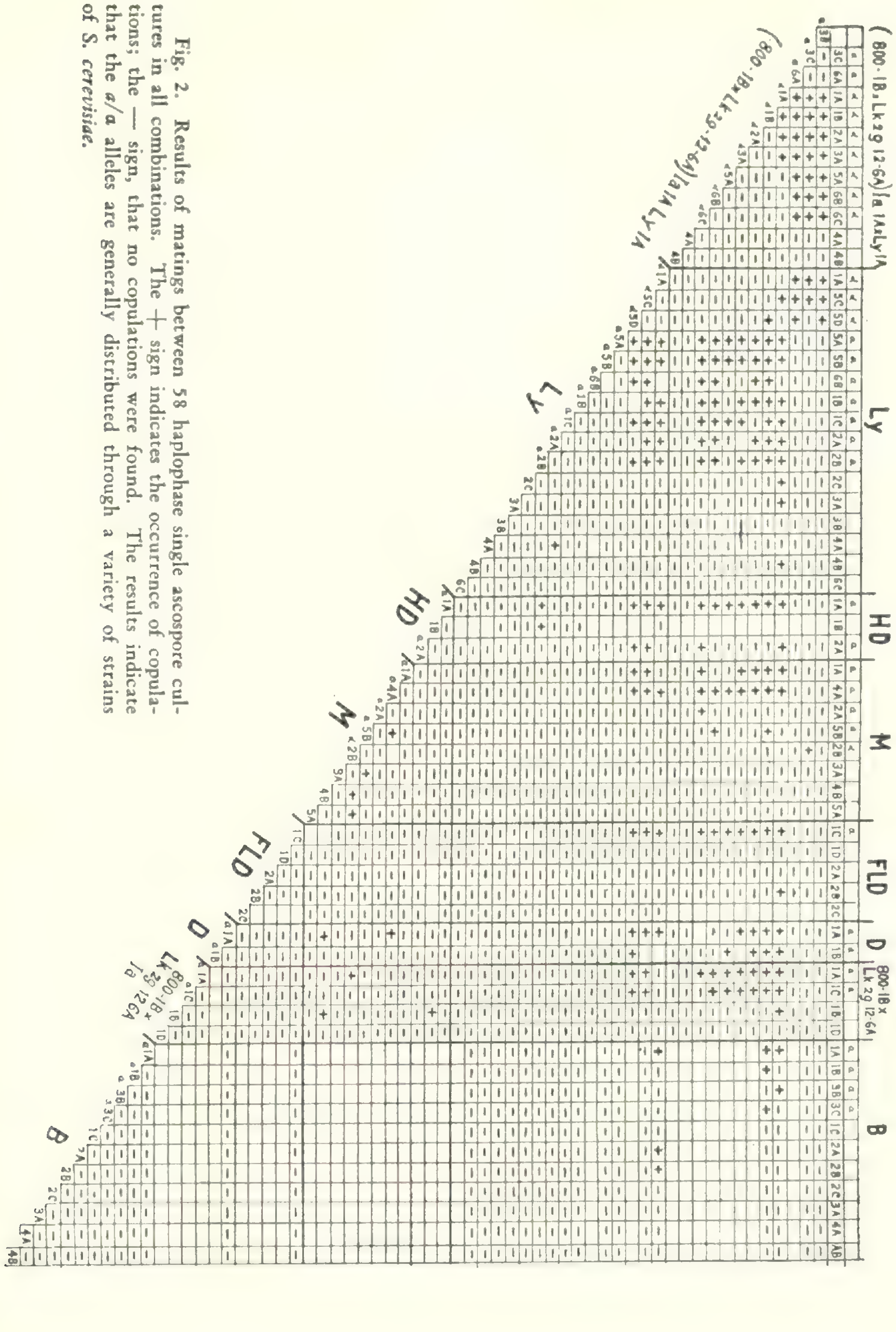

Fig. 2. Results of matings between 58 haplophase single ascospore cultures in all combinations. The + sign indicates the occurrence of copulations; the — sign, that no copulations were found. The results indicate that the *a*/*a* alleles are generally distributed through a variety of strains of *S. cerevisiae*.

Three belong to mating type *a* and seven belong to mating type *α*. Two were sterile. Copulations invariably occurred when an *a* and *α* culture were mated, and haplophase 1A produced illegitimate matings with three other *α* type cultures. The Ly strain was also generally quite fertile. Three *α* type haplophases and seven *a* type haplophases were found in this culture. Only once did an *a* x *α* mating fail to produce fusions. When the "(800 x L) x L" haplophases were mated with the L haplophases, a high degree of fertility was demonstrated, with only seven failures out of forty-eight tests. With this strain also, culture 1A produced illegitimate diploids. This highly fertile culture was also able to mate with 2C, 3A, and 4B, which were incapable of producing fusions with any other culture with which they were tested. When the *a*-haplophases of the L and the "(800 x L) x L" hybrid were outcrossed to the other strains of yeast, only three hybrids were produced in several hundred matings, but outcrossing with the *α* strains was much more successful and resulted in a large number of hybrids. This occurred in spite of the fact that the HD, M, FLD, D, and B cultures were apparently quite infertile among themselves. It appears, therefore, that the *α* strains from this line can be successfully outcrossed to produce hybrids with other strains.

These results demonstrate that the *a*/*α* alleles obey the standard rules of Mendelian inheritance, and that other genes may apparently act as modifiers of mating type, generally resulting in reduced fertility.

CYTOPLASMIC ADAPTATION OF AN ILLEGITIMATE HYBRID

The illegitimate diploids are genetically stable forms because they sporulate rarely and, if transferred frequently in broth, will not sporulate at all. The failure to sporulate eliminates segregation as a cause of variation. Furthermore, diploids are practically free from spontaneous mutations because each locus is "covered" by a dominant normal allele. We have studied adaptation to a specific environment, using an illegitimate diploid. Adaptation to a carbohydrate-peptone mash which contained an unknown substance that inhibited yeasts was studied. The first transfer made from malt medium to this carbohydrate-peptone mash grew very poorly, but adaptation to the new medium always occurred on the second serial transfer.

The malt medium (M) contained 10 per cent malt extract, 0.5 per cent dextrose, 0.5 per cent dried yeast, 1 per cent $CaCO_3$, 3 per cent agar. The carbohydrate-peptone mash medium (C) contained 0.8 per cent sucrose, 0.7 per cent nitrogen-containing solids, 1 per cent $CaCO_3$, 3 per cent agar. M agar is a relatively complete medium which supports an abundant growth of uniformly large colonies. Adaptations of different types of yeast to these media have been reported (Lindegren and Lindegren, '43b). On the first transfer to C agar only a small percentage of cells survives, and the variations in colony-size on this agar are not due to genetic differences. Figure 3 shows the results of plating serially on M and C media.

One of the large colonies from an M plate was suspended in water, and equal

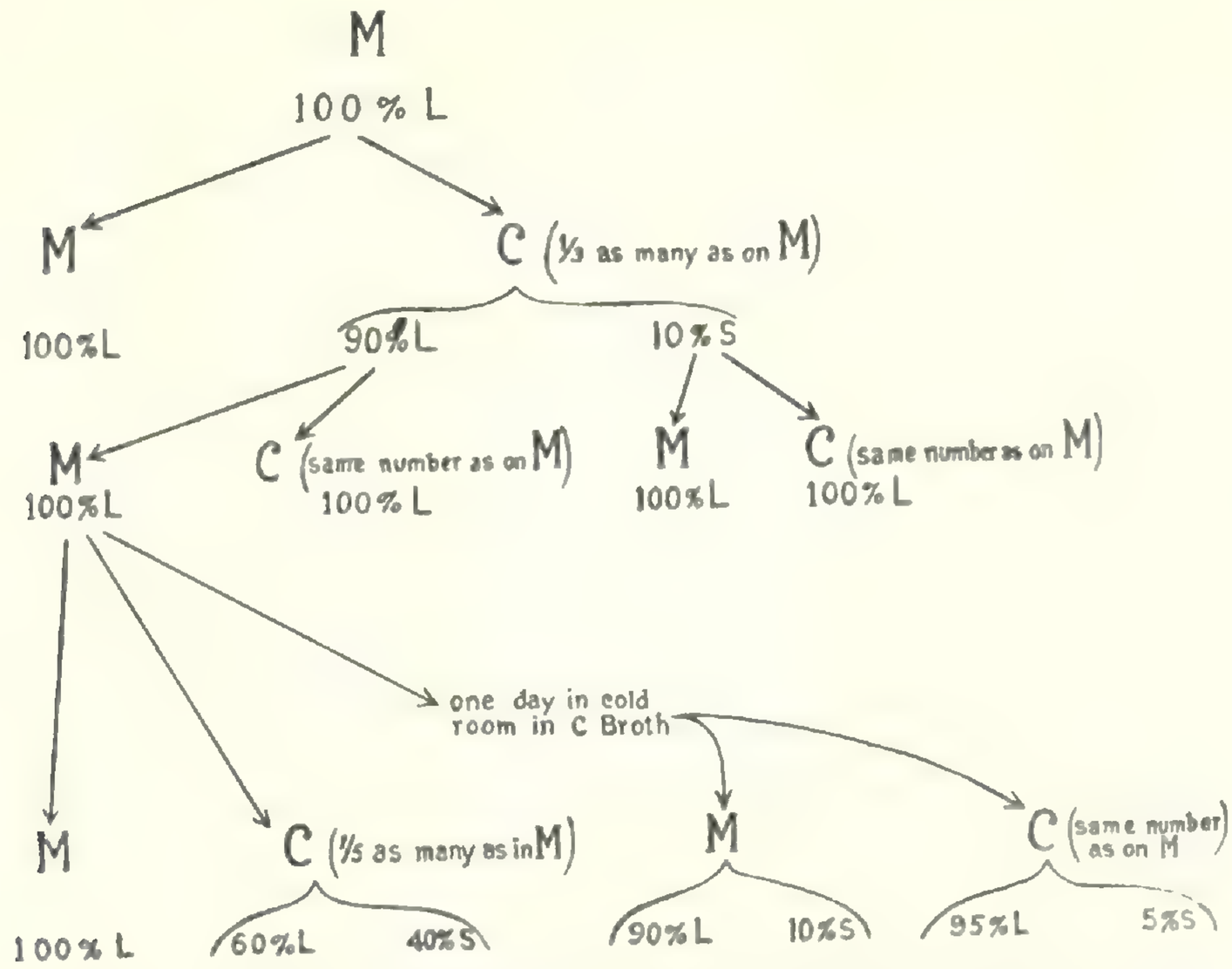

Fig. 3. Diagram showing adaptation of an illegitimate diploid (in which mutations are only rarely expressed) to an unfavorable medium.

amounts of the suspension planted on M and C plates. All the colonies appearing on the M plate were uniformly large. Only about one-third as many colonies appeared on the C plate, and of these 90 per cent were large and 10 per cent were small. In all transfers a large number of cells die; from 10 to 30 per cent of the cells transferred from M to M usually produced colonies, but the mortality is always greater on the first transfer from M to C than on transfers from M to M. Since the genotype is stabilized any selection must be for some difference, independent of the genotype.

A suspension was made of a large colony growing on a C plate, and equal amounts of the suspension plated on M and C plates. The same number of colonies appeared on both plates and all were full size. This proved that the survivors on the C plate were adapted to growth on C agar. A suspension of a small colony gave the same result. The fact that there is no detectable difference between the large and small colonies on C plates indicates that the small colonies are merely slower in development. If a colony is delayed in development, the staling effect of the more rapidly developing colonies on the medium will prevent it from attaining full normal size.

Equal amounts of a suspension from one of the large colonies on an M plate (descended from a colony on a C plate) were plated on M and C plates. Only one-fifth as many colonies appeared on the C plate as on the M plate and both large and small colonies were found. Therefore, cells growing on a C plate (which have become adapted to C agar) lose this adaptation by a single transfer to M agar. This confirms the fact that the first transfer to a C plate did not select genotypes.

It was also possible to adapt the cells to C agar by holding them in C broth in the cold room for two days. A large colony from an M plate was suspended in C broth and held two days in a cold room. When samples from this culture were spread on M and C plates a small number of colonies appeared on both media, although the samples of the untreated culture plated directly from M plates to both M and C plates showed only one-fifth as many colonies on the C as on the M plate.

Since the C medium is obviously unfavorable to the cells coming directly from the M medium it seems probable that the former contains some harmful substance. However, interaction between the cytoplasm and the C medium results in an adaptation apparently enabling the illegitimate diploid to produce a metabolite capable of neutralizing this substance, and this metabolite continues to be produced as long as contact with C medium is maintained. This adaptation must be cytoplasmic because no change has occurred in the genotype. The metabolite which neutralizes the C substance may have been absent from the yeast cells or may be merely increased in amount during adaptation to the C medium. This type of non-genic variation constitutes a complication in the analysis of yeast genetics, and experiments must be designed so that it can be distinguished from the variations resulting from segregation and mutation.

Winge and Laustsen ('40) have demonstrated that a cytoplasmic deficiency may occur in yeasts when a nuclear division is not accompanied simultaneously by a cell division. They have assumed that this condition results from a deficiency of chondriosomes. Their phenomenon is apparently quite different from adaptation of the cell to C medium in which an interaction of substrate and cytoplasm is involved.

MENDELIAN INHERITANCE OF AN ADAPTIVE ENZYME

S. cerevisiae is incapable of fermenting melibiose, and its haploid segregants fail to ferment this sugar even after continued growth in broth containing melibiose. This indicates that mutations enabling the yeasts to ferment the sugar either do not occur in this species or else that they are extremely rare. *S. carlsbergensis* is capable of fermenting melibiose, as are all its haploid segregants. This is the principal character upon which *S. cerevisiae* and *S. carlsbergensis* are differentiated. Figure 4 is a pedigree describing the progenies of matings between these two species (Lindegren, Spiegelman and Lindegren, '44). The data were obtained by growing the cultures in a broth tube containing a smaller inverted tube to collect the gas produced by fermentation. Accumulation of gas in the inverted tube is indicated by a plus sign.

Hybrid I was an interspecific hybrid (*cerevisiae* x *carlsbergensis*) made by mixing melibiose-plus and melibiose-minus haplophase cultures. Three diploid cells isolated after this mating were all capable of fermenting melibiose. Eight asci were dissected from interspecific hybrids, and all the haplophase progeny were tested for the ability to ferment melibiose. The results showed that the haplo-

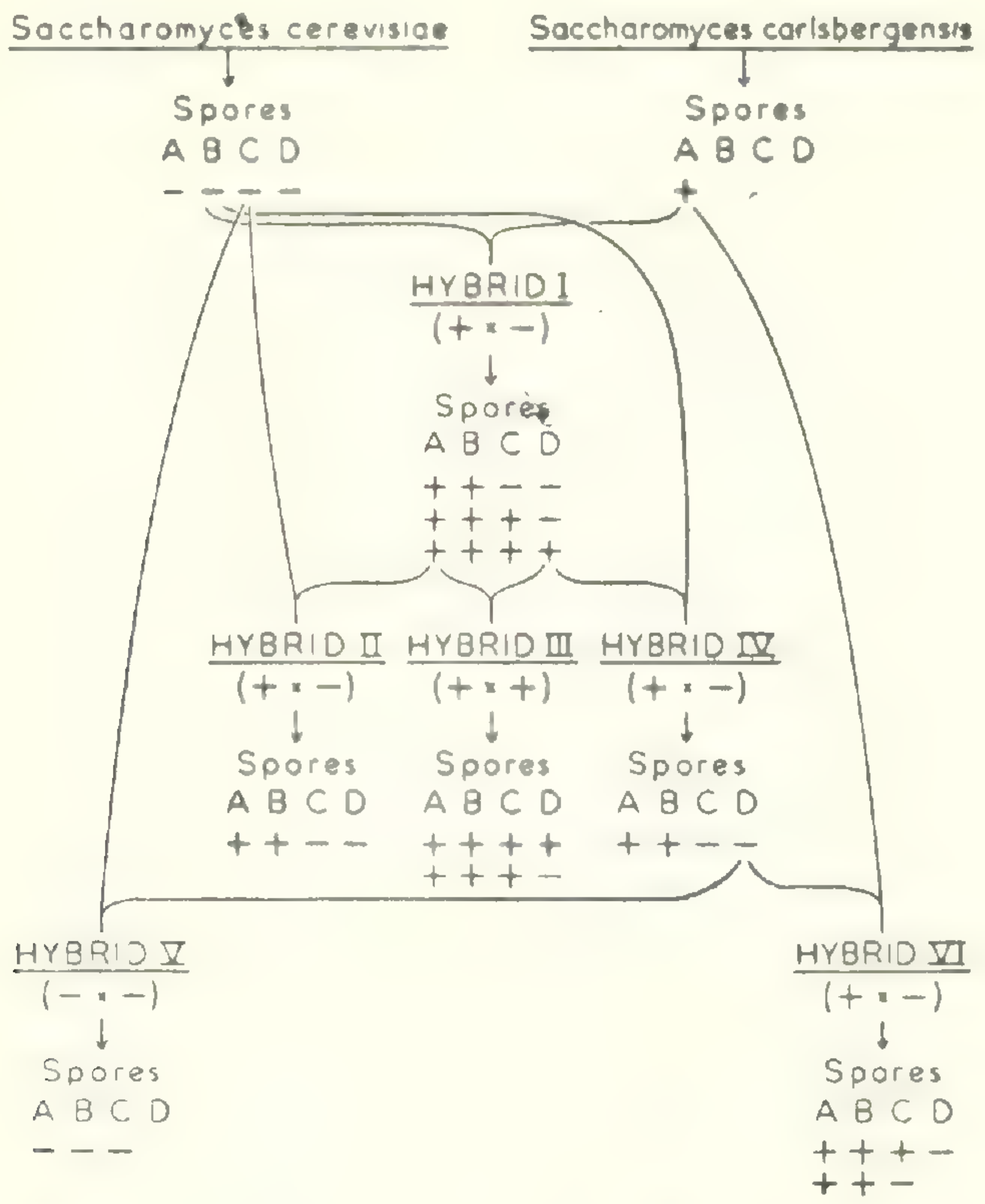

Fig. 4. Fermentation of melibiose by diploid and haploid progeny of *Saccharomyces cerevisiae* x *S. carlsbergensis* hybrids; + sign signifies fermentation of melibiose, — sign, inability to ferment melibiose.

phase cultures from three asci were melibiose +; two asci produced three + and one — culture; and one ascus produced two + and two — cultures. These ratios indicate that more than one gene is involved.

Hybrid II was produced by backcrossing a positive haplophase culture from an ascus producing four + cultures with a negative haplophase culture from S. *cerevisiae*. A regular Mendelian segregation of the progeny shows that the haplophase carried a single gene capable of controlling melibiose fermentation.

Hybrid IV was made by backcrossing a second positive haplophase culture from the same ascus to a negative haplophase culture from S. *cerevisiae*. In this case, a regular Mendelian segregation again shows it also carried a single gene.

Hybrid III was produced by mating the two positive cultures, each of which carried a single gene. Analysis of hybrids II and IV proved that each of these cultures carried a single gene controlling melibiose adaptation. If these genes were alleles, all the haplophase progeny of the hybrid should ferment melibiose. Since two of the twenty haplophase segregants failed to ferment melibiose, the original culture of S. *carlsbergensis* must have contained two different non-allelic loci controlling melibiose fermentation.

Hybrid V was made by backcrossing a negative haplophase segregating from the hybrid to a negative haplophase from S. *cerevisiae*. The three haplophase progeny were all negative.

Hybrid VI was made by backcrossing the same negative culture to a positive haplophase of S. *carlsbergensis*. Five of seven haplophase progeny fermented melibiose, while two failed. This finding, together with the results obtained in hybrid V, confirms the fact that the original haplophase culture of S. *carlsbergensis* possessed two genes controlling melibiose fermentation.

This pedigree is of especial interest because Dr. Spiegelman was able to show that the fermentation of melibiose is under the control of an adaptive enzyme (Kärstrom, '38). Twelve critical cultures were tested in the Warburg apparatus to determine whether fermentation occurred immediately or whether adaptation to the substrate was required, i. e., whether fermentation only occurred after a period of exposure to melibiose. In each case it was found that an adaptive enzyme was involved. The adaptation time was not the same for each strain, which agrees with previous work (Spiegelman and Lindegren, '44), but the time for each is specific and is reproducible under standard conditions.

In the inverted-tube method, part of a clone is seeded into the broth containing melibiose as the carbohydrate source and allowed to grow. Since every mutation in a haplophase population becomes functional immediately, a positive test might not mean that the original clone possessed the fermentative capacity. Selection of a mutant produced during growth in the melibiose solution may have occurred (Spiegelman, Lindegren and Hedgecock, '44). The Warburg tests of the twelve critical cultures excluded this possibility by the fact that in the Warburg apparatus adaptation occurred in a stationary population. If a stationary population exposed to melibiose acquires the ability to ferment the sugar, it can only be due to an interaction between the existing cells and the sugar. Cells which have been adapted to ferment melibiose lose this ability when removed from the substrate and have to be readapted to use it fermentatively.

MAINTENANCE AND INCREASE OF MELIBIOZYMASE IN THE ABSENCE OF THE SPECIFIC GENE

In the preceding experiments on adaptation to melibiose the first contact with the substrate occurred when the culture was transferred to a fermentation tube containing melibiose. A second series of experiments (Spiegelman, Lindegren, and Lindegren, '45) showed that if contact with melibiose were maintained during the growth of the haplophase cultures, during copulation, during growth on the presporulation agar, and during spore formation, all the segregants from heterozygous diploids, such as hybrids II and IV, carrying a single pair of genes, were able to adapt to melibiose fermentation. However, two of the melibiose-plus cultures from each ascus completely lost their ability to ferment melibiose when vigorously dissimilated. This proves that melibiozymase was transferred from the cytoplasm of the heterozygous hybrid which had been maintained on melibiose to the cytoplasm of the haplophase segregants which did not carry the specific gene. Furthermore, the melibiozymase was maintained in the segregants which carried the melibiose-plus gene by an interaction between melibiozymase and melibiose. There-

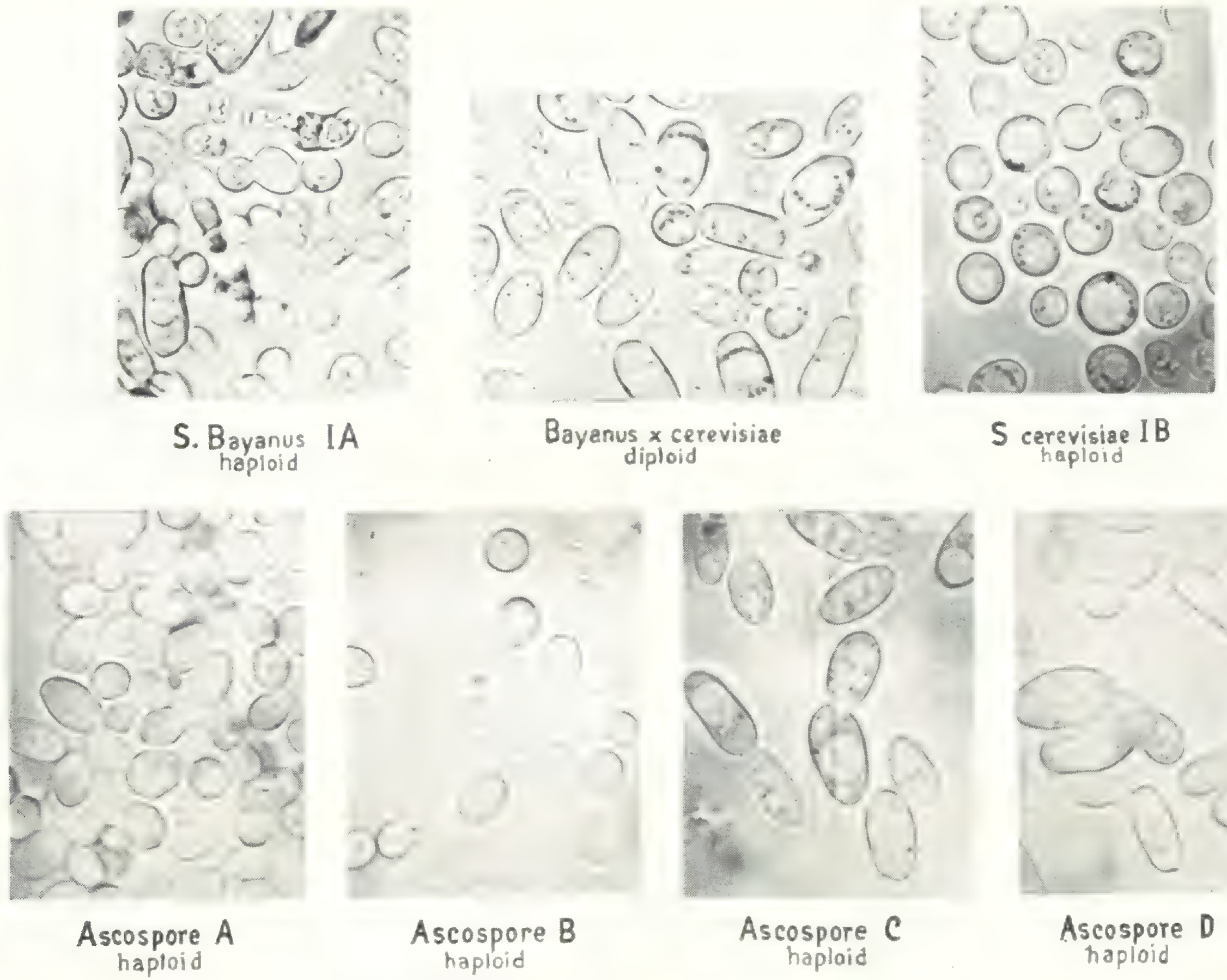

Fig. 5. Photographs of the haplophase cells of *Saccharomyces Bayanus* and S. *cerevisiae* and the diplophase cells of the interspecific hybrid. A, B, C, and D are the haploid cells grown from the four spores of a single ascus of the hybrid.

fore, melibiozymase is a self-perpetuating cytoplasmic entity which is gene-initiated, but the quantitative level of melibiozymase below the maximum depends on an interaction between melibiose and the enzyme and is independent of the gene.

Since S. *carlsbergensis* is homozygous for two pairs of genes which produce melibiozymase, there are four loci in the diplophase of this organism capable of producing this enzyme. There are four corresponding recessive alleles in S. *cerevisiae*, which is probably the most cosmopolitan and best established yeast species. It seems improbable that a successful wild type yeast should carry four functionless genes.

INHERITANCE OF ADAPTATION TO GALACTOSE

The fermentation of galactose by S. *cerevisiae* is due to an adaptive enzyme similar to that produced by S. *carlsbergensis* for the fermentation of melibiose. Since S. *Bayanus* is incapable of fermenting galactose, hybrids between it and S. *cerevisiae* make it possible to study the inheritance of galactose adaptation. There is one advantage in this particular case, namely, that S. *Bayanus* produces large cylindrical cells both in the haplophase and diplophase, providing an additional genetical marker. The hybrid between the large cylindrical (L) gametes of S. *Bayanus* and the round (l) gametes of S. *cerevisiae* produced a large cylindrical (L) diplophase, proving that the *Bayanus*-type cell is dominant. One difficulty is that our culture of S. *Bayanus* sporulated only rarely and only one ascospore of a very large number that was isolated grew. The fact that many of the single ascospore cultures of the hybrid produced viable four-spored asci considerably complicated the genetical analysis. It is notable as an evidence of hybrid vigor that the original hybrid and the progeny all sporulated very abundantly in spite of the poor sporulation of the original S. *Bayanus*.

Figure 6 is a pedigree showing the progenies of a hybrid between S. *Bayanus* and S. *cerevisiae*. All of the haplophase cultures from hybrid IV fermented galactose (+). Half of the single ascospore cultures had large cylindrical (L) cells like S. *Bayanus* and half resembled haplophases of S. *cerevisiae* (l). The large cylindrical *Bayanus*-type cultures fermented galactose more slowly (L + slow) when studied by the inverted-tube technique than did the *cerevisiae*-type (l + fast) cells. The slow fermentation of some of the cultures growing in the fermentation tubes was probably due simply to the slower growth of the *Bayanus*-type segregants.

An analysis of some of the single-ascospore cultures which produced four-spored asci showed that the eight ascospores obtained from two asci isolated from diploid XIII (illegitimate IV-1A) were unable to ferment galactose. This proves that ascospore IV-1A did not carry the gene controlling galactose fermentation but was able to ferment galactose because galactozymase had been carried over cytoplasmically, just as melibiozymase had been carried over in the previous experiments. Our earlier experiments had shown that galactozymase, like melibiozy-

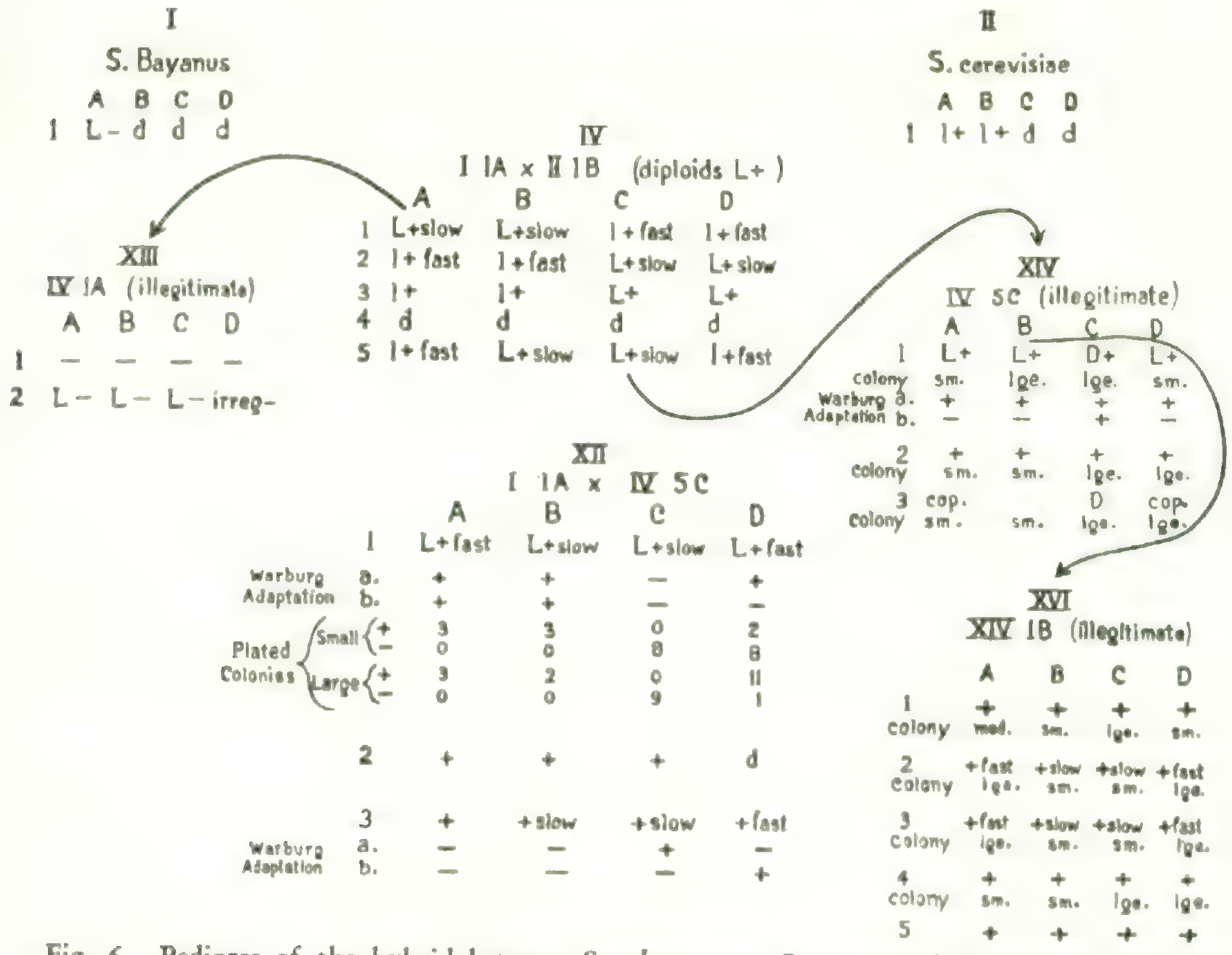

Fig. 6. Pedigree of the hybrid between *Saccharomyces Bayanus* and *S. cerevisiae* and the progeny from it. Discussed in text.

mase, is also substrate-dependent (Spiegelman, Lindegren, and Hedgecock, '44; Spiegelman and Lindegren, '44).

Diploid XIV (IV-5C illegitimate) was L+ and produced viable four-spored asci, and all the single ascospore isolates from it were capable of fermenting galactose. Warburg tests for adaptation to galactose revealed that although all four cultures from ascus 1 adapted within 5 hours on the first trial, three failed to adapt within this period on a second trial. Three asci were dissected from this illegitimate diploid, and each ascus yielded two small- and two large-colony cultures. This suggests that the illegitimate diploid was heterozygous and indicates that copulations in the single ascospore culture had occurred after mutations made the haplophase heterogeneous. Some of the segregants were poorly viable degenerate cells (D) and some produced an abundance of copulations (cop) in the haplophase cultures. These characteristics suggest a similarity to the cytoplasmic deficiencies found in illegitimates by Winge and Laustsen.

Diploid XVI (XIV-1B illegitimate) is the second inbred illegitimate generation derived from IV-5C. Two large- and two small-colony isolates were obtained from each ascus, indicating that the second generation is heterozygous like the first. All the isolates ferment galactose in the inverted-tube tests, and this was confirmed by a second test showing that the original culture, IV-5C, carried the + gene from *S. cerevisiae* together with the *Bayanus*-type cell.

Three asci were dissected from hybrid XII (an L segregant, IV-5C, carrying

the + gene, backcrossed to S. *Bayanus* I-1A, L—). All four ascospores from ascus 1 produced *Bayanus*-celled cultures capable of fermenting galactose. However, when they were grown on sucrose and then tested five hours in the Warburg for adaptation to galactose only A and B proved adaptable in both tests within this period. When all four haploid cultures were plated on agar and single colonies fished and tested for the ability to ferment galactose, A and B produced only fermenting cultures while most of the colonies fished from C and D failed to ferment galactose. These facts indicate that ascus 1 was heterozygous for the +/— alleles.

THE CYTOGENE HYPOTHESIS

These experiments on adaptation show that the ability of different yeasts to adapt themselves to specific substrates is due to a cytoplasmic mechanism. No genetical analysis was available in the case of the adaptation of the illegitimate diploid to C medium, but it was possible to show that genes control the adaptation to melibiose by making hybrids between S. *carlsbergensis* and S. *cerevisiae*, and similarly that genes control the ability to adapt to galactose by making hybrids between S. *cerevisiae* and S. *Bayanus*. In the latter experiments it was clear that although genes initiated the production of the adaptive enzymes, adaptation only occurred by interaction of the cytoplasm of the cells with the specific substrate; and furthermore, the adaptive enzyme, once it had been formed, was self-perpetuating in the presence of the substrate. This was further confirmed in both cases by showing that the adaptive enzyme could be transmitted through the cytoplasm and maintained in cells without the gene.

I propose to call adaptive enzymes of this type *cytogenes*. The fact that a period of exposure to melibiose must occur before the melibiozymase is produced

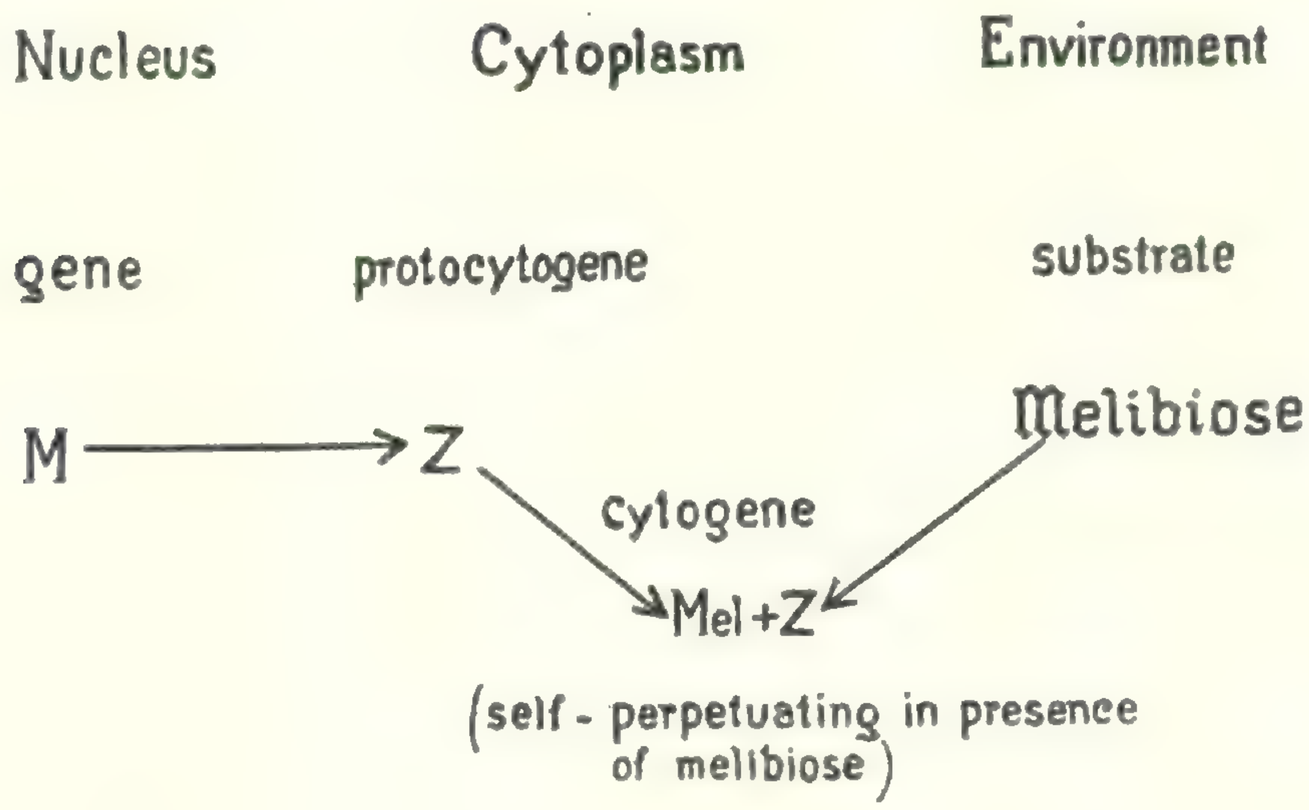

Fig. 7. Diagram explaining the cytogene hypothesis.

suggests that the original product is a relatively non-specific substance which is transformed into melibiozymase when it is "imprinted" by melibiose. The original relatively non-specific substance I propose to call the *protocytogene*. This concept is shown diagrammatically in fig. 7.

If the melibiose-plus gene produces a protocytogene which becomes a specific cytogene, melibiozymase, by being "imprinted" by the melibiose molecule, it is possible that the same locus may be responsible for the production of other cytogenes as well. The original gene-product which becomes specific by contact with the melibiose molecule might presumably become differently specific on contact with some other molecule. Genes are "enzyme factories," but each gene may not necessarily be restricted to the production of a single enzyme. The possibility that a single gene may produce a variety of cytogenes may be a different phenomenon from the one first described by Dobzhansky ('27) as the "manifold effects of a single gene."

DISCUSSION

Darlington ('44) has named certain self-perpetuating cytoplasmic entities which seem to be relatively independent of the genome *plasmagenes*. The cytogene differs fundamentally from the plasmagene, for the former, as defined above, is gene-initiated and substrate-dependent. However, a cytogene might possibly be transformed into a plasmagene by a metabolic mechanism which would synthesize the appropriate molecules within the cell. For example, if *S. cerevisiae* synthesized melibiose, melibiozymase could be maintained permanently in the hybrids as a constitutive enzyme. Such a mechanism might arise by mutation. Therefore, plasmagenes and cytogenes might be phylogenetically related in the following sequence: gene → cytogene → plasmagene.

Darlington has suggested that plasmagenes may evolve into viruses by mutation; however, this implies that plasmagenes are relatively independent entities more or less at the gene level. The preceding discussion suggests that most plasmagenes may be highly dependent on internal substrate for perpetuation rather than relatively independent as Darlington has suggested. The fact that the plasmagenes reproduce exclusively in a specific cytoplasm may mean that their actual existence depends upon contact with some specific type of molecule peculiar to that specific cytoplasm rather than on general "good" growing conditions in the cytoplasm. However, viruses seem to be relatively independent on the substrate and to resemble genes much more than either plasmagenes or cytogenes. Viruses might arise directly from genes rather than from plasmagenes. I ('38) have presented an hypothesis suggesting that viruses may evolve from genes by passage through an insect vector which may have some advantages over the hypothesis suggested by Darlington.

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BIOCHEMICAL GENETICS OF NEUROSPORA

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The production by irradiation of mutant strains of *Neurospora* (Beadle and Tatum, '41) has provided material for the cooperative attack by genetical and biochemical methods on the problem of the mechanism of gene action (Tatum, '44, Horowitz *et al.*, '45, Beadle, '45a). The genetic approach has shown that of the mutant strains so far investigated, both the morphological and biochemical ones are differentiated from normal by single genes. Genetic methods based on crosses and on the formation of heterocaryons have been developed for establishing the allelic or non-allelic nature of specific genes, and for determining the relative dominance of particular biochemical genes (Beadle and Coonradt, '44). The genetic analysis of mutant strains should in time provide data for the location of the mutant genes on chromosome maps. Already quite a few genes have been so located on one or another of the seven chromosomes of *Neurospora* recently demonstrated by McClintock (unpublished).

The biochemical investigations have supported the view that genes controlling biosyntheses of vitamins and amino acids and other biologically important substances in *Neurospora* act through their primary effect in determining the specificity or the production of enzymes involved in carrying on individual steps in the biosyntheses. The mutant strain is perhaps characterized by total or partial failure of enzyme synthesis. Another possibility would be the production of a modified enzyme with altered specificities, which is as a result either inactive or less effective than the normal enzyme in catalyzing the required reaction. If the general concept of a biosynthesis as a sequential series of enzymic reactions is correct, a number of consequences may be predicted which can be tested experimentally. If a given gene affects only one enzyme and therefore only one biochemical reaction, each enzymic step should be controlled by a different gene. Or conversely, two non-allelic genes controlling even the same synthesis must affect different biochemical reactions in that synthesis. It should also follow that an intermediate compound preceding a genetically blocked reaction should be inactive, while one following this point in the biosynthesis might be expected to show the same order of activity as the end product.

The results of investigations have so far supported these predictions. At least seven different genes are known to be involved in the synthesis of arginine. (Srb and Horowitz, '44). Of these, four are concerned in unknown reactions leading to the synthesis of ornithine, two in the conversion of ornithine to citrulline, in which two biochemical steps have been suggested (Krebs, '36), and only one in the conversion of citrulline to arginine, a reaction involving only one obvious step. At least two genes are concerned in the synthesis of tryptophane, one in the production of anthranilic acid and one in the conversion of anthranilic acid

to indole (Tatum, Bonner and Beadle, '44). The latter is then converted to tryptophane by condensation with serine (Tatum and Bonner, '44), a reaction for which no mutant gene has yet been found in *Neurospora*. In the synthesis of thiamin one gene governs the synthesis of thiazole, and another the condensation of thiazole with pyrimidine (Tatum and Bell, unpublished). Another gene is known which apparently controls the synthesis of pantothenic acid from its components, β-alanine and pantoyl-lactone, either one or both of which are inactive for the mutant strain.

A third possible consequence of the original assumptions would be the accumulation of an intermediate the further conversion of which is blocked by the gene mutation. A few examples of this accumulation of intermediate products in *Neurospora* mutant strains are known. The *indoleless* strain 10575 which can form tryptophane from indole but not from anthranilic acid actually produces this latter compound, which must therefore be an intermediate in tryptophane synthesis in *Neurospora* (Tatum, Bonner and Beadle, '44). *Thiazoleless* 18558 produces vitamin pyrimidine, while *thiaminless* 9185 produces both thiamin intermediates but cannot bring about their coupling (Tatum and Bell, unpublished). *Pantothenicless* 5531, which requires intact pantothenic acid, synthesizes both β-alanine and pantoyl-lactone (Tatum, unpublished). Not only has the accumulation of intermediates of known constitution been shown to result from particular gene mutations, but also the production of intermediates of unknown nature. The isolation and identification of these should give some insight into as yet unknown biochemical mechanisms of certain other syntheses. Such an intermediate is produced by *cholineless* 47904 but has choline activity for strain 34486 in which the biosynthesis is blocked at an earlier step (Horowitz, unpublished). Another instance is the production of a nicotinic acid precursor by strain 4540 which is capable of replacing this vitamin for another mutant strain (39401) (Bonner, unpublished). This nicotinic acid precursor instead of accumulating is, under some conditions, further metabolized, apparently with the production of an inactive, intensely colored yellow pigment. An inactive purple-colored compound is apparently formed as the result of a reaction involving an intermediate in the synthesis of adenine (Mitchell, unpublished).

Unfortunately, the accumulation of active intermediates in mutants of *Neurospora* seems to be the exception rather than the rule. In many cases this seems to be due to their lability, which results in the further metabolizing of these products as rapidly as they are formed. Other difficulties in the isolation and identification of these substances are the small amounts produced and the narrow range of cultural conditions under which they can be shown to accumulate. Nevertheless, the results of these investigations of mutant strains suggest that in each a single reaction is blocked, and are consistent with the hypothesis of a one-to-one relation between gene and chemical reaction, through specific enzymes.

A few mutants have been found in which more complex reactions or require-

ments have been indicated. Cases in which the activity of the single essential substance is increased by the addition of a second substance can be interpreted as secondary effects, due to biochemical relations not necessarily connected with the blocked biosynthesis. Examples of this are the sparing action of methionine on *cholineless* (Horowitz and Beadle, '43) and possibly that of thiamin on *pyridoxinless* (Stokes, Foster and Woodward, '43). One well-established actual double requirement resulting from a single gene mutation is that of the two amino acids, isoleucine and valine. The close biochemical relation of these two makes plausible the assumption that their biosyntheses involve either a common precursor or a common enzymatic reaction (Bonner, Tatum and Beadle, '43). This interpretation is consistent with the hypothesis that a one-to-one relation exists between the gene and a given enzyme and primary reaction.

Another instance of a double requirement is known, the basis of which is not so easily interpreted. In strains 17084 and 1090 single-gene mutations apparently block the synthesis of both thiamin intermediates, thiazole and pyrimidine (Tatum and Bell, unpublished). Since there is no obvious biochemical similarity in these compounds the interpretation of the action of the genes concerned on the basis of a single reaction is difficult. One possible interpretation is that the synthesis of only one component is blocked, and that the inactive intermediate which is formed then combines or reacts with the other component or its precursor, thus resulting in an actual deficiency in both. An exogenous supply of both compounds is apparently completely active for these mutant strains. The results of further study of these strains will be of the utmost importance in connection with the general validity of the proposed one-to-one relation of gene and enzyme.

Two fairly common and possibly related phenomena have been met with in *Neurospora* as well as in other micro-organisms. These are cases in which a requirement for a specific substance is altered or dispensed with, the result of prolonged incubation in deficient media, "adaptation" (Bonner, Tatum and Beadle, '43), or as an immediate response to altered cultural conditions. In general, it has been found in *Neurospora* that in both instances the genetic constitution of the modified strains is unaltered. There are two possible explanations for these phenomena. One, suggested for *pyridoxinless* by Stokes *et al.* ('43), is that the gene mutation has resulted in a limitation of the physiological conditions under which the synthesis can be performed by a given mutant strain. This could imply the production by the mutant of an enzyme with more restricted capacities. The other possibility is that there may be alternative mechanisms for carrying on certain syntheses or certain steps in a synthesis, and that these different mechanisms may normally function under different physiological conditions. In this case the gene mutation has resulted in the failure of only one synthetic mechanism. The search for mutants of this type has led to the discovery of a number which require particular substances only under definite conditions, especially at temperatures over 28° C. The substances required by these "temperature" mutants include riboflavin, adenine and uracil (Houlahan and Mitchell, unpublished).

It is possible that the phenomenon of adaptation is essentially analogous to alterations in synthetic capacities in response to external environmental changes. Adaptation could result from either (1) the formation of an adaptive enzyme capable of carrying on an alternative synthetic reaction, the enzyme possibly formed in response to the slow accumulation of the intermediates (substrate), or (2) the lag in adaptation could be due instead to a slow modification in the internal cellular environment, a change eventually leading to the functioning of an alternative reaction, or of the original reaction under the modified conditions in the cells. Further experimental evidence may permit a decision as to whether or not these phenomena involve different reactions. The results should have a direct bearing on the validity of the one-to-one relation of gene and reaction.

The pleiotropic manifestations of certain genes in other organisms may at first glance seem difficult to reconcile with the one-to-one gene to enzyme concept. A possible solution is that relating such effects to multiple functions of the primary gene product (Beadle, '45b). It is possible that certain instances in *Neurospora* may be of value in analyzing some effects of this nature at the biochemical level. In a number of biochemical mutant strains, the primary deficiency is accompanied by a specific sensitivity to other compounds, lacking in other mutant strains and in the wild type. The best analyzed and most striking instance in *Neurospora* is the specific inhibition of *lysineless* by arginine (Doermann, '44). This action of arginine has been interpreted as an inhibition of the utilization of the required lysine by the arginine supplied. The utilization of endogenous lysine in the wild type is not interfered with by exogenous arginine. The different effect of arginine in the two cases may be due to differences in the metabolism of exogenous and endogenous lysine. Other instances also suggest the existence of differences between the physiological action and therefore of the metabolism of exogenous and endogenous materials of biological importance. For *tryptophaneless* mutant strain 10575 indole has a greater molar activity than has tryptophane. This apparently results from the more rapid destruction of added tryptophane than of the tryptophane formed *in situ* from indole. Thiazole has a three- to four-fold greater antipyrithiamin activity than does thiamin (Tatum, unpublished). If pyrithiamin inhibits the utilization of thiamin as suggested by Woolley and White ('43) and by Sarett and Cheldelin ('44), thiamin synthesized in the cell from thiazole must be more effective in antagonizing pyrithiamin than is exogenous thiamin. Houlahan and Mitchell (unpublished) have found with a mutant strain which requires riboflavin at higher temperatures that the growth response to riboflavin is very strongly inhibited by lumichrome, and somewhat by lumiflavin, neither of which inhibits the growth of other strains. These results again suggest differences in the metabolism of endogenous and exogenous substances. The many examples of amino acid inhibitions and antagonisms reported with bacteria and the specific inhibitions noted in *Neurospora* mutant strains, all indicate the complexity of interactions of substances in the living cell, and suggest some of the difficulties to be met with in interpreting data

on growth requirements. These interrelations and interactions may arise in part from differences in the biochemical fates of substances of endogenous and exogenous origin, and may provide a biochemical basis for the explanation of certain types of multiple gene effects.

In conclusion, the results so far obtained with *Neurospora* support the hypothesis that genes concerned in biosyntheses, and probably all genes, act in a primary way by determining the specificity of, or in controlling the production of enzymes. The results also support the view that a one-to-one relation exists between gene and enzyme. At present it seems likely that any apparently multiple gene effects in *Neurospora,* when completely analyzed biochemically and genetically, will be found to be due to common primary reactions, or to secondary interactions not directly related to the action of the mutant gene under consideration.

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GENETIC ASPECTS OF CHANGES IN *STAPHYLOCOCCUS AUREUS* PRODUCING STRAINS RESISTANT TO VARIOUS CONCENTRATIONS OF PENICILLIN

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Fastness or resistance to penicillin and to sulfa drugs is known to occur in strains of bacteria which are sensitive to these chemicals; and in numerous instances on record this new property has been produced *in vitro* under experimental conditions. It is known that the degree of resistance can readily be increased by growing bacteria in a medium containing increasingly higher concentrations of such chemicals, and that the resistant strains so developed retain the property of resistance. My interest in this problem was to obtain quantitative data regarding the origin of resistance and to determine whether or not a genetic interpretation could be given to them. The preliminary summary of these results which has been published (Demerec, '45) indicates that the change to penicillin-resistance in *Staphylococcus aureus* is a genetic change comparable to gene mutation.

Experimental procedure.—The penicillin used in these experiments came from a lot of E. R. Squibb and Sons' preparation of sodium salt of penicillin. This was dissolved in phosphate buffer of pH 6, kept in a refrigerator, and added to the culture medium whenever required by the experiments.

In all experiments a single strain of *Staphylococcus aureus* was used—a culture of which, carrying the number 313, had been obtained from the Northern Regional Research Laboratory in Peoria, Illinois. This same strain has been designated as one of the two international standards for assaying penicillin (Veldee, Herwick and Coghill, '45). In order to reduce the genetic variability of the stock, three cultures were made on agar slants by inoculation from a single colony, and, after 24 hours of incubation, were placed in a refrigerator and used daily as the source of inoculum for all experiments.

Bacteria were grown in a broth medium that did not contain penicillin. They came in contact with the penicillin only when tests for resistance were made. Then certain numbers of bacteria were placed in Petri dishes and mixed with a nutrient agar medium containing the desired concentration of penicillin. These cultures were incubated at 37° C. for 48 hours, and after that period of time bacterial colonies appearing in the medium were counted. The long incubation period was necessary because submerged colonies grow much more slowly in a medium containing penicillin than in one without penicillin.

Results obtained by this method are repeatable; similar numbers of surviving colonies were observed when similar samples of bacteria were taken from the same culture and were plated in a medium containing a certain concentration of penicillin.

Resistance of stock strain.—The strain of *Staphylococcus* used in these experiments was affected by various concentrations of penicillin in the manner shown graphically in fig. 1. The curves given in this figure, representing the results of five experiments, show numbers of survivors when bacteria were grown in penicillin. These six curves are very similar to one another; the heavy line drawn through them represents an average curve.

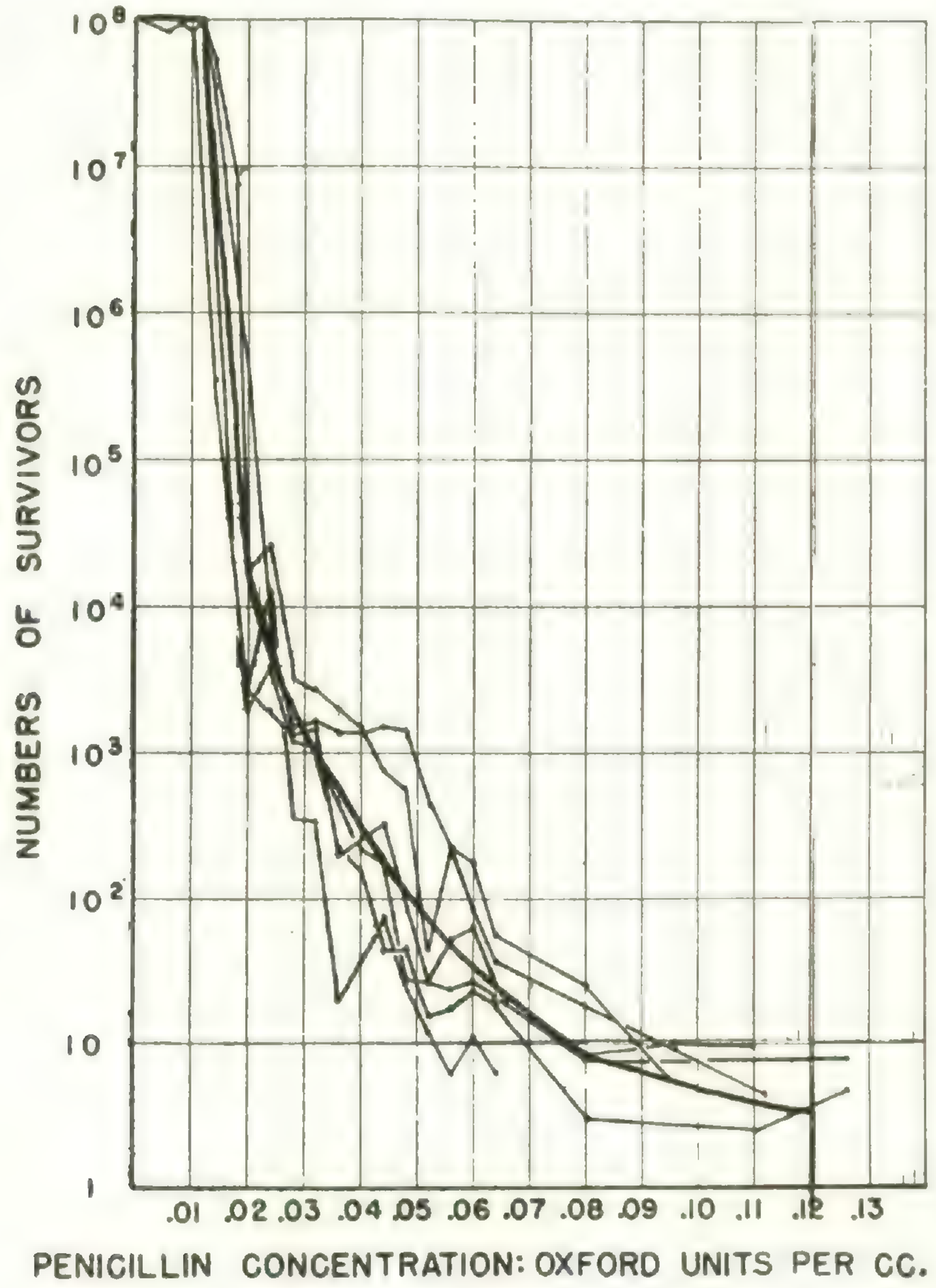

Fig. 1. Numbers of surviving *Staphylococcus aureus* after plating in nutrient agar containing various concentrations of penicillin. The six light curves represent data from six experiments, and the average curve is drawn as a heavy line.

It is evident from fig. 1 that penicillin does not affect these bacteria until a concentration of 0.012 Oxford unit per cc. is reached. That seems to be a threshold concentration for our strain of bacteria. If the concentration is increased by only 0.002 unit over the threshold, the number of surviving bacteria is reduced to 10 per cent. Increase by another 0.002 unit reduces the survivors to 1 per cent;

another similar increase brings the number of survivors down to 1 per 1000; and when a concentration of 0.1 unit per cc. is reached there are on the average only 5 survivors per 100,000,000 bacteria.

The next problem that arose in this study was to find out why some bacteria survive and form colonies in the medium containing penicillin while a great majority of their sister bacteria are eliminated. For this purpose, 32 strains were established—each of them isolated from a single colony that had survived a concentration of 0.064 unit per cc. In order to avoid the possibility of their being members of one clone, each of these colonies was taken from a different experiment. At that concentration (0.064 unit), there are only about 25 survivors per 10^8 bacteria. Tests made with these 32 strains showed that all of them were more resistant to penicillin than was the original stock strain. Survival curves indicated that the threshold for the effectiveness of penicillin had shifted (from the concentration of 0.012 unit per cc. which was the threshold for the original strain) to a region around 0.064 unit per cc. in these selected strains. This result justified the conclusion that survivors in the medium containing 0.064 unit of penicillin per cc. lived because they were resistant to that concentration.

TABLE I

RESISTANCE OF STRAINS ISOLATED FROM COLONIES SURVIVING VARIOUS CONCENTRATIONS OF PENICILLIN

Concentration units/cc.	Number of strains	Resistance higher than parent stock	Resistance similar to parent stock
0.064	32	32	0
0.024	20	18	2
0.022	50	26	24
0.018	54	12	42
0.016	53	10	43

Similar tests were made with 20 strains isolated from cultures containing 0.024 unit of penicillin, with 50 strains isolated from 0.022-unit cultures, 54 strains from 0.018-unit cultures, and 53 strains from 0.016-unit cultures. Results of these tests are summarized in Table I. Of the 20 strains from 0.024-unit cultures, 18 were more resistant than the stock strain, while two had the same degree of resistance as the stock strain. Among the strains isolated at the 0.022 concentration, 52 per cent were resistant; and among those isolated at lower concentrations about 20 per cent were resistant. It is evident that a portion of the survivors on concentrations near the threshold lived not because they were resistant to these concentrations but for some other reason. A possible explanation for the appearance of these survivors is as follows: that occasionally the strength of the

penicillin concentration may be reduced in minute sectors of the medium, owing to some environmental factor, and that in the regions near the threshold concentration such reduction may be sufficient to permit the growth of nonresistant bacteria.

Figure 2 gives survival curves for twelve resistant strains isolated at random at various concentrations of penicillin. The heavy line is the survival curve of the stock strain. Arrows pointing toward it indicate the concentrations at which resistant strains were isolated, and each arrow may be matched up (i. e., solid line, broken line, or dotted line) with the curve of the corresponding resistant strain. It is evident that strains isolated from colonies surviving higher concentrations of penicillin tend to be more resistant than strains isolated from colonies surviving lower concentrations.

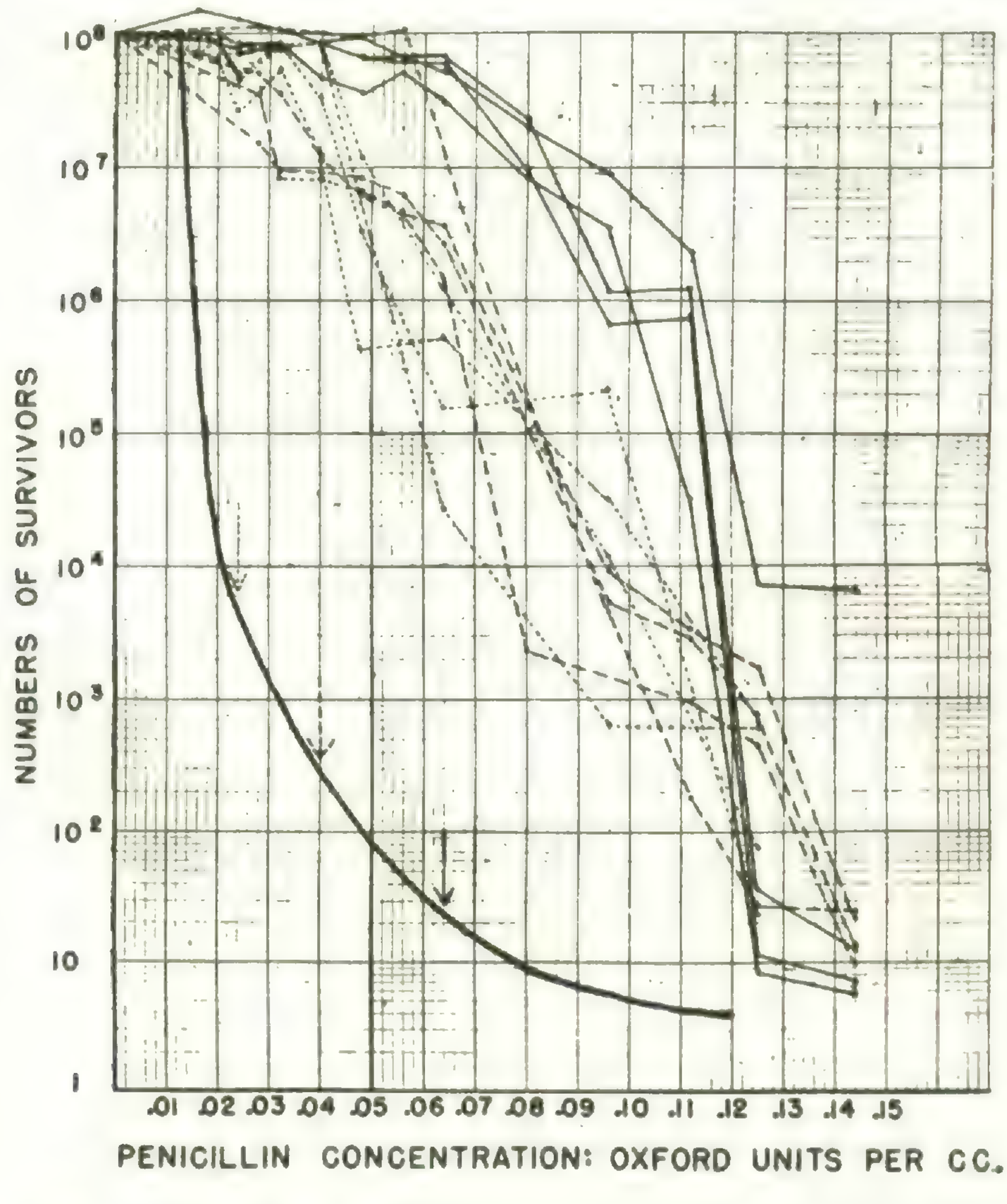

Fig. 2. Heavy line—survival curve for the stock strain; light lines—curves for strains established from colonies surviving the concentrations of penicillin indicated by the arrows.

Inheritance of resistance.—The next problem to be investigated was whether resistance is an inherited characteristic, which persists, or a temporarily acquired feature. Ten strains, isolated from colonies surviving in a concentration of 0.064 unit of penicillin per cc., were passed through 20 transfers in broth; and their survival curves were determined at the beginning of the experiment, at several intervals during the experiment, and at the end of the experiment. The fact that there was no appreciable difference between these curves indicates that the degree of resistance did not change during 20 transfers. Similar results showing the persistance of resistance have been obtained by Schmidt and Sesler ('43) and by Spink, Ferris and Vivino ('44). Thus it is probable that the increase in resistance to penicillin is hereditary, and that it is a stable feature.

Origin of resistance.—The next problem to be investigated dealt with the mechanism of the origin of resistant strains. Here two possibilities were considered: (1) that resistance is an acquired characteristic brought about through some sort of interaction between the bacteria and the penicillin when they are in contact with each other, and (2) that resistance is an inherited characteristic, which originates through mutation and whose origin is independent of penicillin treatment. In the latter case, resistant mutants should occur at random, in a small fraction of a population; and, since a certain concentration of penicillin eliminates all nonresistant individuals, the resistant ones would be selected out from the population by the treatment.

To distinguish between these two possibilities, a modification of the method developed by Luria and Delbrück ('43) in their study of changes in bacteria from bacteriophage-sensitivity to bacteriophage-resistance was used. If the resistance is induced through interaction between the bacteria and penicillin when they are in contact with each other, it would be expected that approximately similar numbers of resistant bacteria would be obtained when samples containing similar numbers of bacteria are plated in nutrient agar containing a certain concentration of penicillin, irrespective of the origin of these samples. The situation would be quite different in the event that the origin of resistance is mutational. In such case, one would expect to obtain similar numbers of resistant colonies only in samples taken from the same culture. If, however, each of the samples comes from a separate culture, and mutations occur at random, then one would expect to obtain a large number of resistant colonies from cultures in which mutation happened to occur early in the growth of the culture and a small number of resistant colonies from cultures in which mutation happened to occur late, assuming that resistant bacteria grow more or less like the normal. If resistance originates by mutation, then, the variation in number of resistant bacteria between samples taken from separate cultures should be much greater than between samples taken from the same culture.

One of the experiments to test these two possibilities was conducted as follows: From the same broth dilution, containing about 300 bacteria per cc., 30 tubes

were prepared with 0.3 cc. of material each, and one tube with about 15 cc. of the material. At the same time, 20 samples of 0.3 cc. each from the same dilution were plated in the medium containing 0.064 unit per cc. of penicillin, to determine if any of the samples contained resistant bacteria. None was observed; and therefore it was reasonable to assume that each culture was started with an inoculum consisting of susceptible bacteria only. Cultures were incubated at 37° C. for about 18 hours, and during that time the number of bacteria increased to about 2×10^8 per cc.; that is, in the 30 small cultures, from about 100 to about 6.6×10^7. The entire contents of each of the 30 tubes were plated in a Petri dish with 0.064 unit of penicillin per cc. of the culture medium; and 20 samples of 0.3 cc. each were taken from the large tube and were similarly plated with the medium containing 0.064 unit of penicillin per cc. In each of these 50 platings about 6.6×10^7 bacteria were placed in medium containing an identical concentration of penicillin; therefore, if resistance develops through interaction between bacteria and penicillin, one would expect to find on each plate a similar number of resistant colonies. However, if resistance originates through mutation, then one would expect that the 20 samples taken from the same culture would give similar numbers of resistant colonies, while an appreciable degree of variation in number of resistant colonies would be expected among samples taken from the different cultures. The results (Demerec, '45) show very slight variation in number of resistant colonies among the 20 samples taken from one culture. The extreme variants are 16 and 38; the average number of colonies per culture is 28.9; the variance is 39.8, χ^2 is 22.7, and P is 0.3. On the other hand, the number of resistant colonies per sample taken from independent cultures varies greatly. The extreme variants are 9 and 839, the average is 120, the variance is 42,718, χ^2 is 10,670, and the probability that such a distribution may be due to sampling is extremely small.

The results of this experiment, therefore, favor the assumption that resistance to certain concentrations of penicillin originates through mutation, and that resistant bacteria may be found in any large population. In this case, the proportion of resistant bacteria depends on the mutation rate.

Effect of selection on degree of resistance.—The strain of *Staphylococcus aureus*, NRRL-313, is eliminated if grown in a medium containing more than 0.15 Oxford unit of penicillin per cc. As has been mentioned earlier, an average of 25 out of 10^8 bacteria survived the concentration of 0.064 unit per cc. From these, strains more resistant than the original strain were established. In strains developed from survivors on an 0.125 concentration, there were individuals resistant to 0.25 unit; strains from these latter survivors had individuals resistant to 0.5 unit; strains from these included individuals resistant to 4 units; and from these a strain was isolated that was not affected by a concentration of 250 units of penicillin per cc. of the agar medium.

The result of this type of selection on increase of resistance to penicillin is

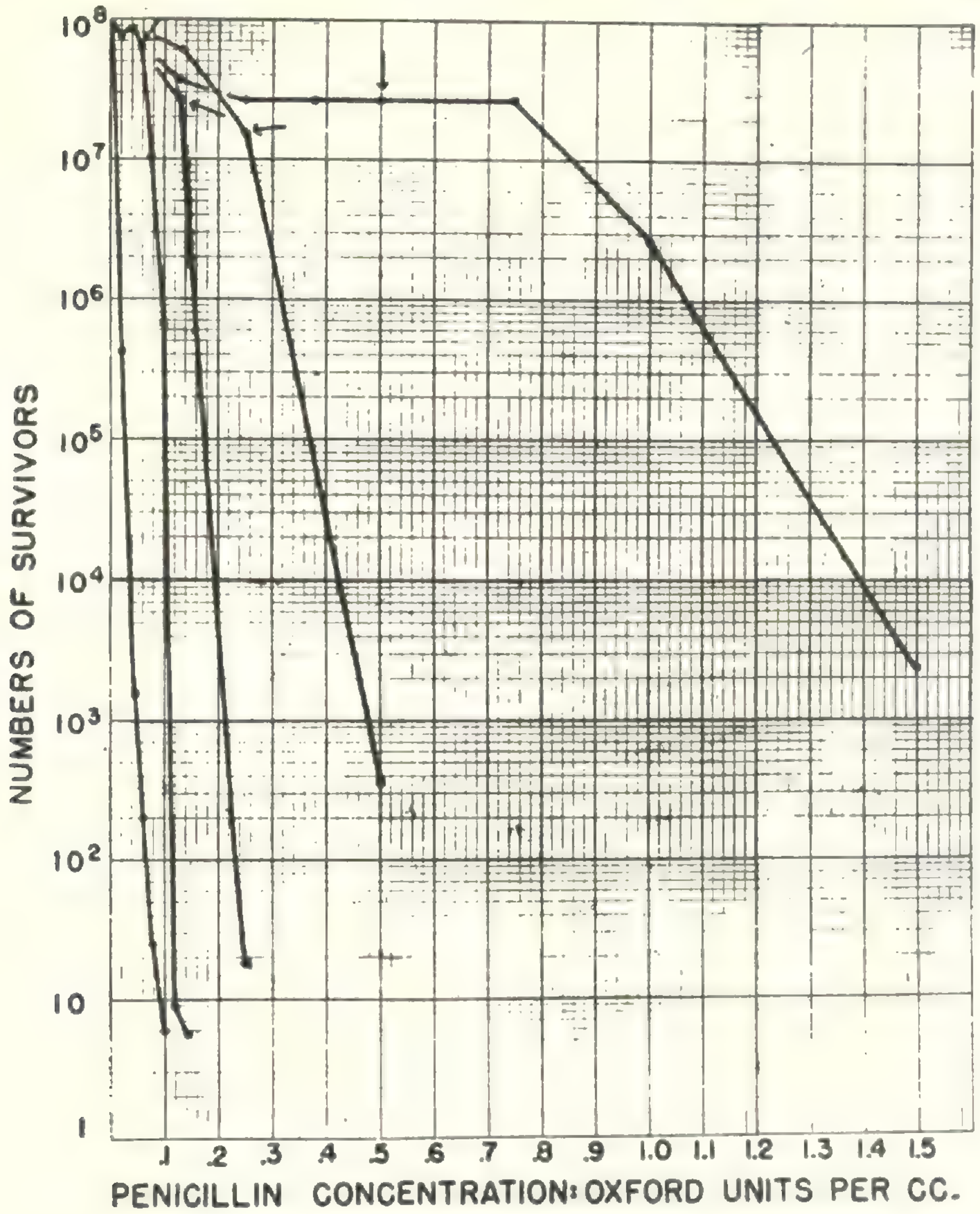

Fig. 3. Numbers of survivors in various concentrations of penicillin, for the stock strain and for four resistant strains developed through repeated selection.

graphically shown in fig. 3. The first curve in this figure represents the number of survivors in various concentrations of penicillin for the stock line of *Staphylococcus;* the second curve represents survivors in a strain isolated from a concentration of 0.064 unit; the third curve shows survivors of a strain isolated from an 0.125 concentration; the fourth is for a strain isolated at an 0.25 concentration; and the fifth for a strain isolated at 0.5 unit. The arrows indicate, on each curve, the concentration at which the strain was isolated. It is evident that the building up of resistance is more rapid with each selection step; the increase appears to be exponential.

Discussion.—The evidence presented here indicates that resistance is a complex characteristic, and that it must involve a number of mutations. If it is assumed that genes are responsible for these mutations, a number of genic changes must be involved. This assumption can readily explain the increase of resistance obtained through selection. A mutation in one of the several

genes determining resistance would produce a line having a low degree of resistance. A mutation in another gene, occurring in this line which already had a degree of resistance, would produce a line (double mutant) whose resistance was higher than the sum of the resistances that would be produced by the two mutations if they occurred separately. The increase in resistance caused by another mutation occurring in the double-mutant line would raise it to a degree greater than the sum of resistances produced by the same three mutations occurring separately; and every successive mutation in a multiple-mutant line would produce a similar effect—that is, an increase in resistance proceeding exponentially with the number of mutant genes involved.

Summary.—In experiments with *Staphylococcus aureus*, strains resistant to penicillin were developed which retained that property after 20 transfers in broth.

Experimental evidence indicates that resistance is not induced by the action of penicillin, but originates as a change comparable to mutation. In any large population of bacteria there are some individuals resistant to certain low concentrations of penicillin. If this population is exposed to the action of such concentrations of penicillin, nonresistant individuals are eliminated while the resistant survive.

Degree of resistance can be increased by selection; and this increase is more rapid with each selection step.

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THE PHYSIOLOGY AND GENETIC SIGNIFICANCE OF ENZYMATIC ADAPTATION[1]

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THE PHENOMENON OF ADAPTATION

Wortmann (1882) showed that certain bacterial species could produce amylase only when grown in the presence of starch. Since these early observations many more of a similar nature have been made. The bacteriological literature in particular contains innumerable instances of so-called "training" phenomena of the most varied kinds. Recent reviews by Karström ('38), Yudkin ('38), Rahn ('38), Stephenson ('39), Linderstrom-Lang ('40), Dubos ('40), and Gale ('43) summarize the available data.

The essentials of the phenomenon may be stated in the following terms: a population of cells placed in contact with some substrate acquires, after the lapse of some time, the enzymes necessary to metabolize the added substrate. The removal of the substrate leads to the disappearance of the enzyme system it evoked.

Karström ('38) designated as "adaptive" those enzymes which are produced as a specific response to the presence of the homologous substrate. Such enzymes were differentiated from the "constitutive" ones which are always formed by the cells of a given species, regardless of the presence or absence of their homologous substrates.

Because of its convenience, Karström's terminology has been widely adopted. In recent years, however, it has become increasingly clear that it is not adequate for the description of the facts. In the first place, the classification raises the obvious difficulty that the ease with which a given enzyme is detected in low amounts will determine the category in which it is placed. In addition, enzymes which have been labeled constitutive undergo wide fluctuations in the presence and absence of their substrate. Thus, the invertase content of *B. coli* rises to 452 (Stephenson, '39) in the presence of sucrose and falls to values lying 12.4 and 39 in its absence. Again the $Q_{glucose}$ value of *B. coli* is about 1,000 for organisms grown in the presence of glucose and about 190 for those grown in lactate medium (Stephenson and Gale, '37). In all carefully examined instances where enzyme-substrate relations are known, substrate has stimulated or stabilized its enzyme. The only claim for independence of enzyme level from its substrate was made by Quastel ('37), who reported that glucose stimulated urease formation whereas urea suppressed it. However, Epps and Gale ('42) reinvestigated the problem and showed that the differences Quastel observed depended on the fact that ex-

[1] Certain of the investigations reported here were aided by a grant from the Penrose Fund of the American Philosophical Society. Grateful acknowledgment is also made to the Department of Zoology of Washington University for the facilities so generously placed at the disposal of the author.

traction was done in the absence of substrate. They found that growth in the presence of urea stabilized the urease content of cells.

While exceptions may be found by future study, there does exist a relatively large group of well-defined enzyme systems which respond positively to their specific substrates. In the case of the "adaptive" enzymes, the response is marked and the enzymes fall to zero or near zero levels in the absence of substrate. These enzymes seem to differ from the constitutive ones solely in their relatively greater instability in the absence of substrate. It seems questionable, from this point of view, whether classification into "adaptive" and "constitutive", implying as these terms do a difference in origin and function, is fruitful or even valid. This same point of view implies that enzymatic "adaptations" are but quantitatively exaggerated instances of a more general phenomenon resulting from the effects of substrates on the synthesis and stability of their enzymes.

While we shall in the present discussion use the term "adaptive" in connection with enzyme formation, it should be emphasized that this is not meant to imply the *de novo* induction by substrate of the enzyme concerned. The term is used here to describe the situation in which an enzyme responds by increasing in the presence of its substrate and decreasing in its absence.

From the standpoint of genetics, enzymatic adaptation has several interesting possibilities. Thus far, attacks on the problem of the nature of gene action has had to depend, for the most part, on the study of the final end products of enzymatic activity. It has generally been assumed that genes determine phenotype by virtue of their control of enzymatic constitution. If this be true, the process of adaptation presents an unique opportunity for examining certain details of gene action. In particular, it is reasonable to hope that such studies could delineate the nature of the control exercised by genes over enzyme activity. It is the purpose of this paper to present some data bearing on this problem.

We shall confine our attention to galactozymase and melibiozymase activities and their variations in yeast cells.

BIOLOGICAL MECHANISMS OF POPULATIONAL ADAPTATIONS

Large numbers of individuals are always involved in adaptation experiments, and it is inevitable that attempts to elucidate further the biological nature of these modifications encounter a basic problem common to all studies of physiological changes in large populations. A comparative biochemical study of large populations always involves over-all populational characteristics. This necessarily introduces difficulties into the interpretations of any observed changes in physiological properties. The mechanisms available to an individual cell for adapting itself to an environmental change are limited by its genome and the physiological flexibility permitted by its particular degree of specialization. When, however, the adaptation of a population of cells is being considered, there must be added to the physiological pliability of its members the genetic plasticity of the group in terms of the numbers and kinds of variants it is capable of producing.

Because of this composite nature of populational adaptability, it is clear that in any given case the same end result can be obtained by any one of the following mechanisms: (1) The natural selection of existent variants with the desired characteristics from a genotypically heterogeneous population; (2) induction of a new (as far as measurements are concerned) enzyme by the substrate in all the members of a homogeneous population; and (3) a combination of natural selection and the action of mechanism (2) on those selected.

It was difficult to resolve these questions with bacteria since genetic control over their populations is not attainable. In two instances, however, a decision on the biological mechanism involved was possible. Lewis ('34) showed that the ability of so-called "trained strains" of *B. coli* to ferment lactose originated through the natural selection of a spontaneous variant which was always present in the original culture in the ratio of about $1:1 \times 10^5$. Stephenson and Stickland, ('33) were able to demonstrate the formation of hydrogenlyase in the presence of formate in non-dividing cultures of *B. coli.*

It is clear that a considerable advantage would be gained if it were possible to study this phenomenon with microorganismic populations whose genetics could be controlled. Aside from the obvious possibility of examining the genetics of the process, the study of its physiology could be enormously simplified. Reproducibility of the measurements would thus be assured and the complications of natural selection, which are always present when dealing with genetically heterogeneous material, could be avoided. The opportunity of using genetically controllable material was provided by the fundamental work of Winge and Laustsen ('37, '38, '39a), in Denmark, and the Lindegrens ('43a, b, c), in this country, on the genetics and life cycle of the yeasts.

THE GENETIC CONTROL OF GALACTOZYMASE FORMATION IN YEAST POPULATIONS

Dienert ('00) was one of the first to describe a well-defined example of populational adaptation in the yeasts. He showed that suspensions of yeast cells could acquire the enzymatic apparatus necessary to ferment galactose when placed in contact with it. Since its discovery by Dienert this particular problem has been investigated by numerous workers. Armstrong ('05) confirmed Dienert's findings and further found that some yeasts were incapable of acquiring this physiological property, no matter how long they were cultured in the presence of galactose. Slator ('08) showed that those yeasts capable of fermenting galactose possess this ability only after they had been acclimatized by culture in its presence. No yeast he investigated was able to ferment this hexose immediatley upon being introduced to a medium containing it. There was always an induction period of variable length connected with the acquisition of this property.

Several attempts were made to decide whether natural selection or a direct interaction between the galactose and the cytoplasm was involved in the appearance of galactozymase. Sohngen and Coolhaas ('24) grew their yeast cultures at

30° C. and measured enzymatic activity at 38° C. to avoid cell division during the measurement of CO_2 evolution. They concluded, from their experiments, that the production of galactozymase parallels the formation of new cells. In addition, they confirmed Kluyver's ('14) findings that at 38° C., at which temperature cell division is completely inhibited, no adaptation takes place. Other investigators also tried to obtain adaptation in the absence of cell division, since this would clearly exclude the operation of natural selection as a causal agent in effecting the change. Euler and Nilsson ('25) and later Euler and Jansson ('27), in a more thorough investigation, tried, without success, to adapt yeast in the presence of 0.5 per cent phenol to inhibit cell division. The failure of the above-mentioned authors to find adaptation in the complete absence of cell division cannot be taken as conclusive evidence that no such phenomenon could exist. Especially is this true in those cases where suppression was obtained by such agents as heat or cellular poisons. It is not unlikely that in cultures where this "ideal" had been reached, the physiological state of the cells was such that their ability to synthesize new enzymes had been lost along with their ability to divide.

Stephenson and Yudkin ('36) concluded from their experiments that the production of galactozymase in yeast cultures need not involve the formation of new cells. This conclusion was based on the observation that the ability to evolve CO_2 anaerobically, from a medium containing galactose, was acquired in a period when the total and viable count remained constant. These findings were apparently in direct contradiction with those of previous workers and in particular of Sohngen and Coolhaas ('24).

Before undertaking any detailed study of the physiology of this adaptation, it was clearly necessary to resolve this difficulty. It is evident from the nature of the problem of populational adaptability that one of the crucial problems at issue is the phenotypic homogeneity or heterogeneity of the starting population. The possibility of attacking the problem from this point of view was provided by the use of known haploid and diploid strains, thus permitting genetic control over the populations being studied.

Two strains of *Saccharomyces cerevisiae*, Db23B and LK2G12, both of which could acquire the ability to ferment galactose when grown in its presence, were selected for study. Strain Db23B, which was known to be a haploid and therefore genetically unstable, was shown (Spiegelman, Lindegren and Hedgecock, '44) to be phenotypically heterogeneous with respect to galactose fermentation. Some individuals in populations derived from this strain could not adapt to ferment galactose, whereas others could. Strain LK2G12, on the other hand, which was known to be diploid, was uniformly homogeneous in that all of its individuals were able to acquire the capacity for the fermentative utilization of galactose on standing in contact with the sugar. The adaptive behavior of these two strains followed what would be expected from the data obtained on their phenotypic

characteristics. Populations of Db23B, starting with a low percentage of the fermenting type, could increase their enzymatic activity only through the mechanism of cell division and the subsequent selection in favor of the galactose fermenters. It was shown (Spiegelman and Lindegren, '44) that the kinetics of adaptation by Db23B populations were in agreement with the natural selection hypothesis. Using the appropriate strains, experiments were performed which sought to duplicate the findings reported by Sohngen and Coolhaas and those reported by Stephenson and Yudkin. The results are summarized in fig. 1.

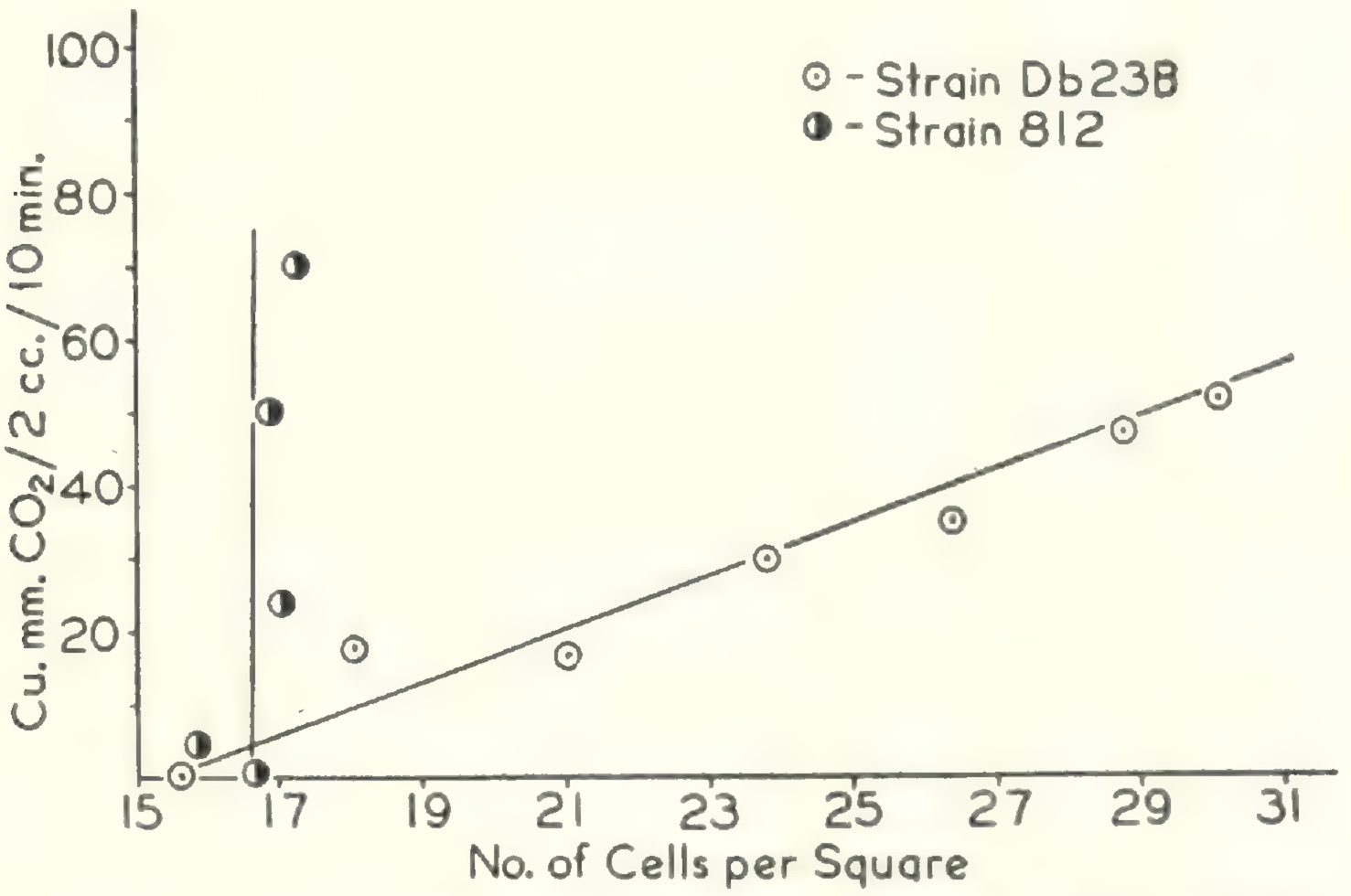


Fig. 1. A comparison of the variation of enzyme activity (expressed as rate of anaerobic CO_2 production) and the number of cells in adapting cultures of haploid (Db23B) and diploid (812) strains.

It is strikingly apparent that populations of the haploid strain, Db23B, increase their enzyme activity by virtue of the new cells arising during the experimental period, and agree with Sohngen and Coolhaas. On the other hand, the measured activity of the diploid 812 population was, in the period examined, virtually independent of cell number. This strain was able to increase its activity from zero to an activity level of 70, while maintaining its population at the same density. The results with this strain thus confirm Stephenson and Yudkin.

From these results it is clear that the contradiction noted is only an apparent one and is probably due to the differences in the genetic background and phenotypic constitution of the strains employed. The conclusion may also be drawn that it is futile to attempt to decide, as some previous authors have tried to do, between the "natural selection" hypothesis and the one of "direct cytoplasmic interaction", as *the* explanation for the production of some one adaptive enzyme. The particular biological mechanism involved in the production of a given enzyme or enzyme system in a population of cells is a characteristic of the strain being examined, rather than of the enzyme system itself. Such questions cannot be answered without referring to the genetic background and stability of the popula-

tion being studied. It is also evident that such strains as LK2G12 and 812 possess three important characteristics of immense value for investigations into the physiology of enzyme synthesis, namely, (1) a genome which permits the synthesis of the enzyme being studied; (2) the genetic stability to insure reproducibility of the physiological characteristics of the populations, and (3) the ability to adapt without cell division.

THE GENETIC BASIS OF INABILITY TO ADAPT TO GALACTOSE FERMENTATION

From the earliest investigations by Armstrong ('05) and Kluyver ('14), the existence of unadaptable yeasts was noted. Subsequent investigations have uncovered many more. As may be seen from a perusal of Stelling-Dekker's ('31) monograph on the sporogenous yeasts, examples of non-fermenters of galactose exist in all the genera.

It did not seem improbable, in view of our experience with Db23B, that the failure to adapt certain of the strains might be due to incomplete utilization of their mutational potentialities. Thus, a non-sporulating population of diploids or one in which some other mechanism existed for the suppression of the haplophase would be unlikely to gain a new character or lose an old one by mutation. This suggested the possibility of attempting to adapt strains, which had been previously labeled as non-fermenters of galactose, by encouraging the production of haploids and thus disturbing the genetic stability of the population. From the point of view of the life cycle of the yeast, this could usually be accomplished by inducing heavy sporulation and allowing germination to occur, thus releasing haploid cells into the population.

Such experiments were performed (Spiegelman and Lindegren, '45) with three yeast types, *Schizosaccharomyces Pombe*, *Schizosaccharomyces octosporus*, and *Saccharomycodes Ludwigii*. All three were investigated by Armstrong ('05), who concluded that they were incapable of adaptation to galactose fermentation. In addition to the fact that they have been studied more thoroughly than other non-fermenters of galactose, they were selected for another advantage, which is of some importance from a comparative point of view. *Sch. Pombe* can, without any difficulty, exist in the haplophase. This is not true of *Sch. octosporus* and still less so for *Saccharomycodes Ludwigii*.

The mechanisms of suppression of the haplophase in the latter two strains differ. While *Sch. octosporus* sporulates with ease, the haploids which result from the germination fuse rapidly to produce diploid cells. When spores from four- and eight-spored asci from the stock culture were planted, they all grew and every single-spore culture thus obtained sporulated on the agar in less than 48 hours. The further spore analysis of this strain indicates that the diploid stock culture was completely homozygous and that, unlike *S. cerevisiae*, the production of viable spores apparently does not depend on the prëexistence of a heterozygous nucleus. The sporulation of single-spore cultures was never observed in the *S. cerevisiae* strains used in the adaptation studies. The fact that it does occur in

Sch. octosporus is an indication of heavy diploidization which is confirmed by direct microscopic observation. Isolated non-fusing haploid cells are rarely seen in suspensions of single-spore cultures of *Sch. octosporus*. This process of immediate fusion effectively suppresses the haplophase, and in these cultures genetic variations come mainly from recombinations. This source of variation would obviously not be effective in populations which are homozygous for the recessive.

The suppression mechanism is even more highly developed in *Saccharomycodes Ludwigii*. Guilliermond ('03) reported that this yeast usually forms four spores without previous conjugation. On germination, however, the spores conjugate within the mother cell, two by two, so that only two vegetative cells emerge from each four-spored ascus. Winge and Laustsen ('39b) confirmed these observations and described the successful isolation of the haplophase by micromanipulative dissection of the ascospore and separation of the four spores before germination. As a result of their examination of the haplophase cultures they concluded that *Saccharomycodes Ludwigii* was a balanced heterozygote. The net result of the germination mechanism is the almost complete suppression of the haplophase under normal conditions.

When heavily sporulating (20 per cent and above) cultures of *Schizosaccharomyces Pombe* were seeded into 8 per cent galactose, 2 per cent glucose media, adaptable populations were recovered. The results are summarized in Table I. Exactly similar experiments with *Schizosaccharomyces octosporus* and *Saccharomycodes Ludwigii* failed. These failures are understandable in terms of the inability of the latter strains to express the mutational potentialities of their haplophases. It might also be noted that adaptable cultures were never recovered from *Sch. Pombe* in the absence of heavy sporulation no matter how long contact with galactose was maintained.

It is clear from these experiments that inability to adapt is in some cases due to the genetic stability conferred by diploidy. It may, however, be doubted whether all that is required for populational adaptation is the breakdown of the genetic stability of the unadaptable strain. It is conceivable that the haplophase of a particular strain might not contain within its mutational potentialities the ability to mutate in the direction of, for example, galactose fermentation. That such indeed could be the case was shown by the isolation of three haploid strains (Spiegelman and Lindegren, '45) which could not mutate towards galactose fermentation although kept in contact with the sugar over a four-month period. During this same period they were, however, throwing off numerous physiological and morphological mutants of various kinds.

As was noted by Lindegren ('45), preliminary experiments on hybrids between S. *Bayanus* and S. *cerevisiae* clearly indicate a typical genetic control of adaptability and non-adaptability to galactose fermentation.

TABLE I

ADAPTATION OF *SCHIZOSACCHAROMYCES POMBE* TO GALACTOSE FERMENTATION

Experiment	Origin of heavily sporulating cultures (20% and above)	Days required for appearance of adaptation
1	20-day broth culture	8
2	20-day broth culture	6
3	24-day agar slant	12
4	Gypsum Block	2
5	Gypsum Block	6
6	Gypsum Block	4
7	Gypsum Block	4

BIOCHEMICAL ASPECTS OF ADAPTATION

Before undertaking an analysis of the genetic implications of the phenomenon, there are certain questions it would be desirable to answer as completely as the data allow. These questions would involve, among other things, the existent evidence for enzyme formation, possible biochemical functions of the induced enzymes, and connection of the adaptation with the over-all metabolic activity of the cell.

Implicit in the discussion presented here, as well as in the entire literature of the so-called "adaptive" enzymes, is the assumption that when a cell is placed in contact with some substrate and acquires, during the course of time, the ability to metabolize the added substrate, a new enzyme must have made its appearance. This assumption stems, of course, from the innumerable observations that every metabolic process requires an enzyme. Direct proof that an enzyme is synthesized would involve its isolation from the adapted cells and is not available in any known case of adaptation. Such proof, in any case, would have to be preceded by a determination of the number and functions of the enzymes induced by the substrate. At present the best that can be offered is evidence of enzyme activity in cell extracts after adaptation. In the case of both galactozymase and melibiozymase, sufficient work has been done to remove any reasonable doubt on the question of enzyme involvement in the adaptation. A delayed penetration into the cell can certainly not be invoked as the explanation of the induction period in galactose fermentation. It was shown (Spiegelman, '45a) that galactose actually enters the cell immediately and is metabolized by a purely aerobic mechanism in the preadaptive period before the fermentative enzymes make their appearance. Further, it has been found earlier (Harden and Norris, '10) that yeast juice and maceration extract prepared from adapted yeasts grown on galactose were able to

ferment galactose. Similar preparations from glucose-grown cultures were inactive. These experiments were repeated and confirmed with our own strains using toluol cytolysates. It is clear from these experiments that something, possessing galactose-fermenting capacity, can be extracted from cells after adaptation which was not there before. Experiments of the same type on cell extracts were performed in adaptations to melibiose fermentation. Here again activity could be demonstrated in the cytolysate only after adaptation was established in the intact cells. It may be noted here that all such extracts were made in the presence of substrate.

Certainly a question of prime importance is the nature of the enzymatic changes necessary for the newly acquired metabolic property. Is a whole new set of enzymes required? Or, is only one or two formed which would transform the sugar into one utilizable by the glucozymase system?

In the case of melibiozymase, it seems most probable that a single enzyme only is formed which splits melibiose into glucose and galactose. An enzyme of this kind has been demonstrated in emulsin preparations by Kuhn ('23), who called it melibiase. Little direct work on this enzyme has as yet been done.

Because of the relative importance of galactose in mammalian physiology, a considerable amount of work has been done on the nature of the enzymatic change which permits a yeast cell to ferment this hexose. Harden and Norris ('10) found that a fermenting mixture of yeast-juice (from an adapted yeast) and galactose reacted with added phosphate in a manner similar to ordinary yeast juice and glucose, although a longer induction period was necessary. The rate of CO_2 formation was accelerated, an extra amount of CO_2 equivalent to the phosphate added was evolved, and the rate then again became normal. The phosphate was converted into an organic form not precipitable by magnesium citrate mixture. Euler and Jansson ('27) showed that when dried adapted yeasts are washed, they fail to ferment galactose. However, such preparations can be reactivated to galactose by adding the co-enzyme prepared from unadapted yeast. They were thus able to exclude the possibility that adaptation is concerned with the modification of the existent cozymase or the formation of a new one. Nilsson ('43) was able to isolate from the products of the fermentation of galactose by a sample of dried adapted yeast, a diphosphoric ester which, in its elementary analysis and specific rotation, closely resembled the hexosediphosphate formed during the fermentation of glucose, fructose and mannose.

In order to explain the formation of the same diphosphate from galactose as from the normally fermentable sugars, Nilsson ('43) suggests that the fermentation of a hexose involved the splitting of the monophosphate ester into a triose and a triose phosphate ester. This would destroy the spatial specificity of the fourth carbon atom in the galactose molecule and the resulting phosphorylated triose could be built up into fructose diphosphate. If this were true, it would be necessary to postulate the formation of only one enzyme in the adaptation to galactose fermentation.

It is true that Meyerhof and Lohmann ('34) have shown the existence of an enzyme (zymohexase) in yeast and muscle preparations capable of converting triosemonophosphoric ester into fructose diphosphate. Nevertheless, experimental support for Nilsson's scheme of fermentation is relatively weak. He assumes that the formation of fructose-di-phosphate is an artifact and that the fermentation proceeds from his postulated split of the hexose monophosphate. He bases this on the observation that fructose diphosphate is fermented much more slowly than fructose. However, this can be explained in terms of the classical Meyerhof-Parnas fermentative scheme on the basis of the unavailability of phosphate acceptors. Thus, Meyerhof and Lohmann ('27) found a CO_2 production of only 25 per cent of the calculated values when maceration extract fermented Robison esters. Warburg and Christian ('39) have shown that the partial dephosphyorylation of 1:3-diphosphoglyceric acid to 3-phosphyglyceric acid by the hexokinase-adenosine diphosphate-hexose system may become limiting in the fermentative process.

Cattaneo ('33) has obtained additional evidence pointing to the convergence of the fermentation paths of galactose and glucose by isolating phosphoglyceric acid from the phosphorylated products formed during the fermentation of galactose by preparations of adapted yeast in the presence of added phosphate, acetaldehyde and sodium fluoride. Grant ('35) reinvestigated this problem and confirmed the work of previous investigators on the role of phosphorylations in the metabolism of galactose by adapted yeast. He was able to establish, with some certainty, that the phosphorylated products which accumulated during the fermentation of galactose are not the esters of this sugar but of glucose and fructose. The hexosediphosphoric ester constituted the major portion of the esterified phosphate. From the monophosphate fraction he was able to isolate trehalosemonophosphate and small amounts of a monophosphate that closely resembled the Robison ester in its properties. Attempts to detect the presence of galactose-phosphate by the methylphenylhydrazine test failed. Perhaps of even greater weight was the fact that he showed that a preparation from adapted yeast, which would ferment galactose, failed to ferment synthetically prepared galactose-6-phosphate. On the basis of this evidence, he concludes that galactose-6-phosphate is not an intermediate in the fermentation of galactose by adapted yeast cells. Of further interest is the evidence he presents that the living yeast cell continues to build up the same polysaccharides when galactose is the sole carbohydrate metabolized as when the carbohydrate is glucose. Hydrolysis of these polysaccharides indicated that they are polymerides chiefly of glucose, and to a lesser extent of mannose and fructose.

Kosterlitz ('43) found that galactose-1-phosphate is fermented by extracts of galactose-adapted yeasts. He found further that, although such extracts fermented glucose at higher rates than galactose, the fermentation rates of galactose-1-phosphate and glucose-1-phosphate were identical. It must be noted, however, that the fermentation rate of the mono-esters was lower than the corresponding

ones for the free hexoses. On the basis of the equality of the fermentation rates of mono-phosphate esters, Kosterlitz postulates the existence of the following equilibrium,

$$\text{Galactose-1-p} \leftrightarrows \text{Glucose-1-p.}$$

However, the same experimental results (unequal rates for the unphosphorylated hexoses, equal but lower rates for the monophosphates) can be explained if either the phosphotase activity or phosphate acceptance were limiting. This would also explain the lower rate of fermentation rate of the esters.

Kosterlitz, on the basis of his experimental results, proposes the following hypothesis of adaptation to galactose fermentation: the formation of two new enzymes is involved. Enzyme (1) phosphorylates galactose at C_1 probably by a system similar to the hexokinase-adenylpyrophosphate systems which phosphorylates glucose and fructose at C_6 (Meyerhof, '35). Enzyme (2) converts galactose-1-phosphate to Robison ester, probably by way of glucose-1-phosphate and subsequent action of phosphoglucomutase or isomerase.

The evidence for the formation of two enzymes stems from the observation that in two out of five samples of dried yeast (Kosterlitz, '43), the yeasts were adapted to ferment glucose-1-phosphate but could not ferment non-phosphorylated galactose.

The bulk of the evidence presented strongly indicates that the adaptive utilization of galactose occurs through the early entrance of the galactose into the glucose metabolic cycle. A non-enzymatic equilibrium (e. g., glucose-1-P $\leftrightarrows$ galactose-1-P) is ruled out by the existence of galactose non-adaptable but glucose-fermenting strains. On the same basis it is clear that the induction period observed in galactose adaptation is not analogous to the lag period observed in glucose fermentation by ordinary yeast brei, which is explained on the basis of a relatively slow accumulation of phosphate esters.

From the studies of the biochemistry of the adapted cell, as well as the stereochemical structure of galactose, it seems most probable that two enzymes at least are formed during the course of the adaptation: One for the phosphorylation of galactose and one for the isomerization of the phosphorylated product into an intermediate of glucose fermentation. In so far as the adaptation is concerned, it would be necessary to postulate that a single substrate can induce the formation of enzymes other than the one for which it is the specific substrate. This, however, raises no real difficulty since, if the enzymes act serially, the product of the first would be the substrate of the second and so on. Thus one substrate could provide a whole series of substrates for a whole series of enzymes.

THE PHYSIOLOGY OF ADAPTATION

In contrast with the relatively vigorous investigation into the biochemistry of the galactose-adapted cell, little has been reported which elucidates the nature of the preadaptive period and the conditions under which the enzyme activity makes its appearance. Certain facts were, however, established by the earlier work.

Attempts to obtain adaptation with non-viable cells or with cells whose physiology has been seriously interferred with by various reagents have uniformly met with failure. The sole exception was the report by Abderhalden ('25) that he had obtained adaptation with dried dead yeast cells. This experiment has never been successfully repeated, and his failure to check the possibility that the adaptation may have been due to the growth of a few surviving cells throws some doubt on the validity of his conclusions. Von Euler and Nilsson ('25) claimed that adaptation will not occur when the cells are suspended in ordinary phosphate containing galactose and they maintained that the addition of "Z" factor was necessary. The earlier experiments of Dienert ('00) contradicted this, since this author did all his experiments with washed cells in ordinary solutions of phosphate.

Our own experience agrees with that of Dienert. All our adaptations were performed with thoroughly washed cells suspended in phosphate solutions of twice-recrystallized galactose. According to these results, the enzyme can be

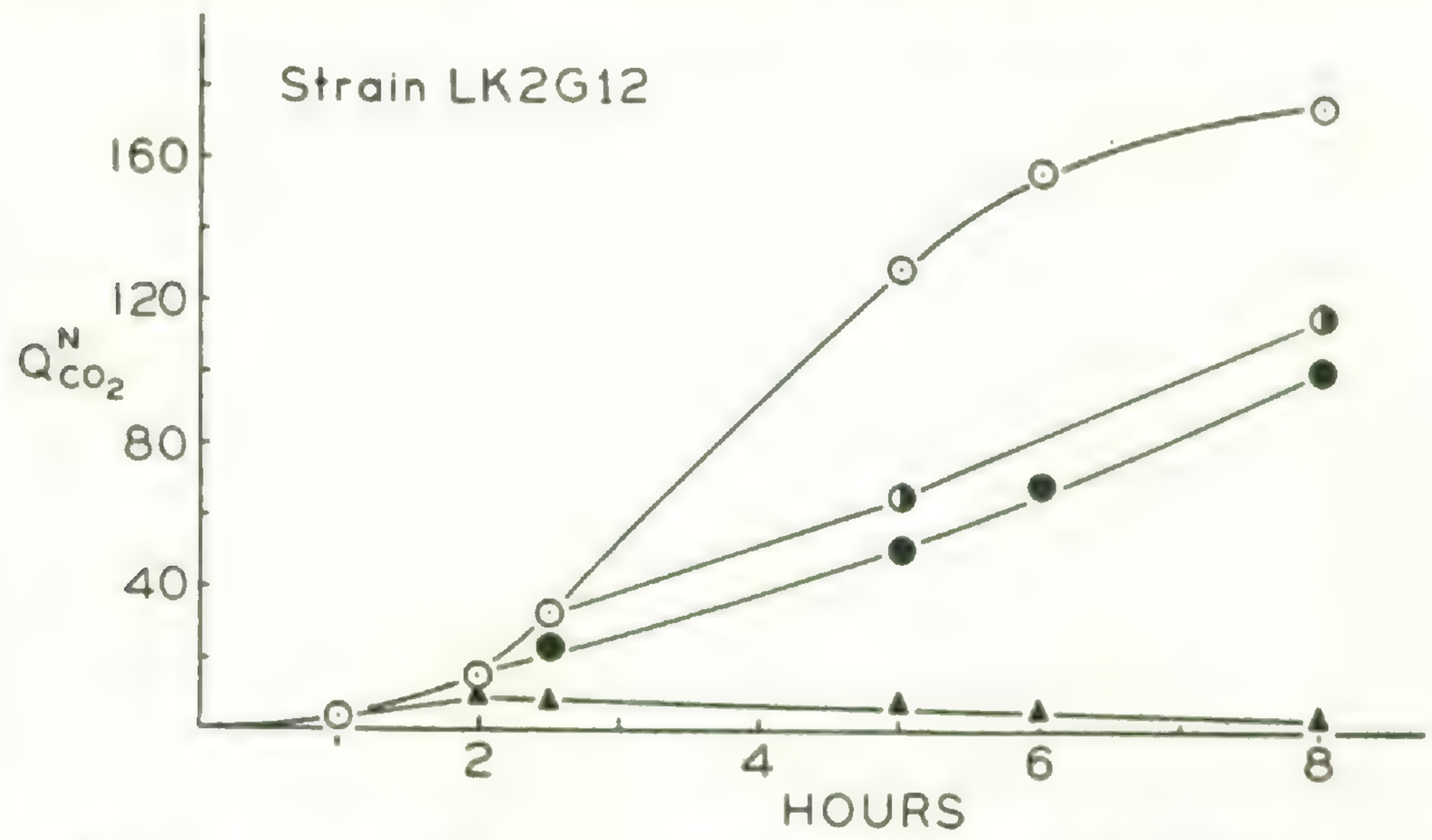


Fig. 2. A test of the ability of cells to increase their galactozymase activity subsequent to aerobic induction periods in contact with galactose. The open circles are the values arrived at for a culture having continuous access to oxygen; the triangles, full-shaded and half-shaded circles represent the subsequent behavior during anaerobiosis.

formed in the absence of an external source of nitrogen. One must conclude, then, that there exists in the cell a source of proteins on which the cell can draw for enzyme formation. It must be noted, however, that the attainable activity level is about half that arrived at if an external source of nitrogen, in the form of ammonium salts or amino acids, is provided (Spiegelman, '45).

Using non-dividing diploid populations, an attempt was made to begin the study of the connection between the synthesis of these enzymes and the over-all metabolism of the cell. Examination of the effect of oxygen (Spiegelman, '45a) revealed that the adaptation was extremely sensitive to oxygen (cf. Stephenson and Yudkin, '36; Schultz, Atkin and Frey, '40). Some strains were found that were completely unable to form galactozymase if they experienced only anaerobic

contact with galactose, while others could form the enzyme anaerobically. However, the rate of anaerobic adaptation was approximately 1/40th of that attained in oxygen. In the case of the strains unable to adapt anaerobically, it was of some interest to determine whether enzyme formation could occur at all under anaerobic conditions. This was done by following the activity anaerobically subsequent to brief periods of aerobic incubation with galactose. The results obtained are given in fig. 2. The open circles are values attained during continuous aerobic incubation with 4 per cent galactose. The anaerobic behaviour of the enzyme activity, following various periods of aerobic contact, is represented by the solid triangles and full and half-shaded circles.

It is seen that at the end of the first hour of aerobic contact, the $Q^{N}_{CO_2}$ value is about 2.8. Under anaerobic contact there is an initial slight rise above this value and a subsequent slow but consistent drop. At the end of two hours of aerobic contact, the $Q^{N}_{CO_2}$ attains a value of 15, and subsequent anaerobiosis does not prevent its increase, although the rate of increase is slower than that obtained in the continual presence of oxygen.

It is clear from these experiments that, while adaptation cannot be initiated anaerobically in these strains, it can proceed under these conditions providing an adequate enzyme activity has been built up. The condition to be met here appears to be that enough enzyme be formed aerobically to utilize the energy content of the galactose molecule when anaerobiosis is established at a rate adequate for further synthesis. We have here the interesting physiological situation where the substrate not only stimulates the formation of an enzyme, but, in addition, acts as the only source of energy for its synthesis. That the energy supply is critical seems clear from two facts. It has been shown (Spiegelman and Nozawa, '45) that for these strains, in common with others (see Stier and Stannard, '35a, b), the endogenous reserves are not fermentable. Further, supplying external fermentable substrate (e. g., fructose and, under certain conditions, glucose) permits (Spiegelman, '45b) the adaptation to take place anaerobically in those strains in which it ordinarily does not occur.

These results suggested that the aerobic adaptation occurred because, under these conditions, the cell could draw on the energy coming from the oxidation of the endogenous reserves for synthetic activity. Experiments were therefore performed to examine adaptation times (time to reach a $Q^{N}_{CO_2}$ value of 100) when the galactose was added at different levels of the endogenous respiration.

The results on one strain are given in fig. 3, in which, for purposes of orientation, the endogenous respiration curve is also diagrammed. It is clear from this figure that up to the zero-rate portion of the endogenous curve little difference in adaptation times is encountered. However, the important point to note is that, although adaptation times increase as the galactose is added further out along the zero-rate portion, nevertheless adaptation occurs. These experiments would seem to indicate that adaptation can take place after all the oxidizable reserves have been exhausted and that there is no apparent source of energy. This

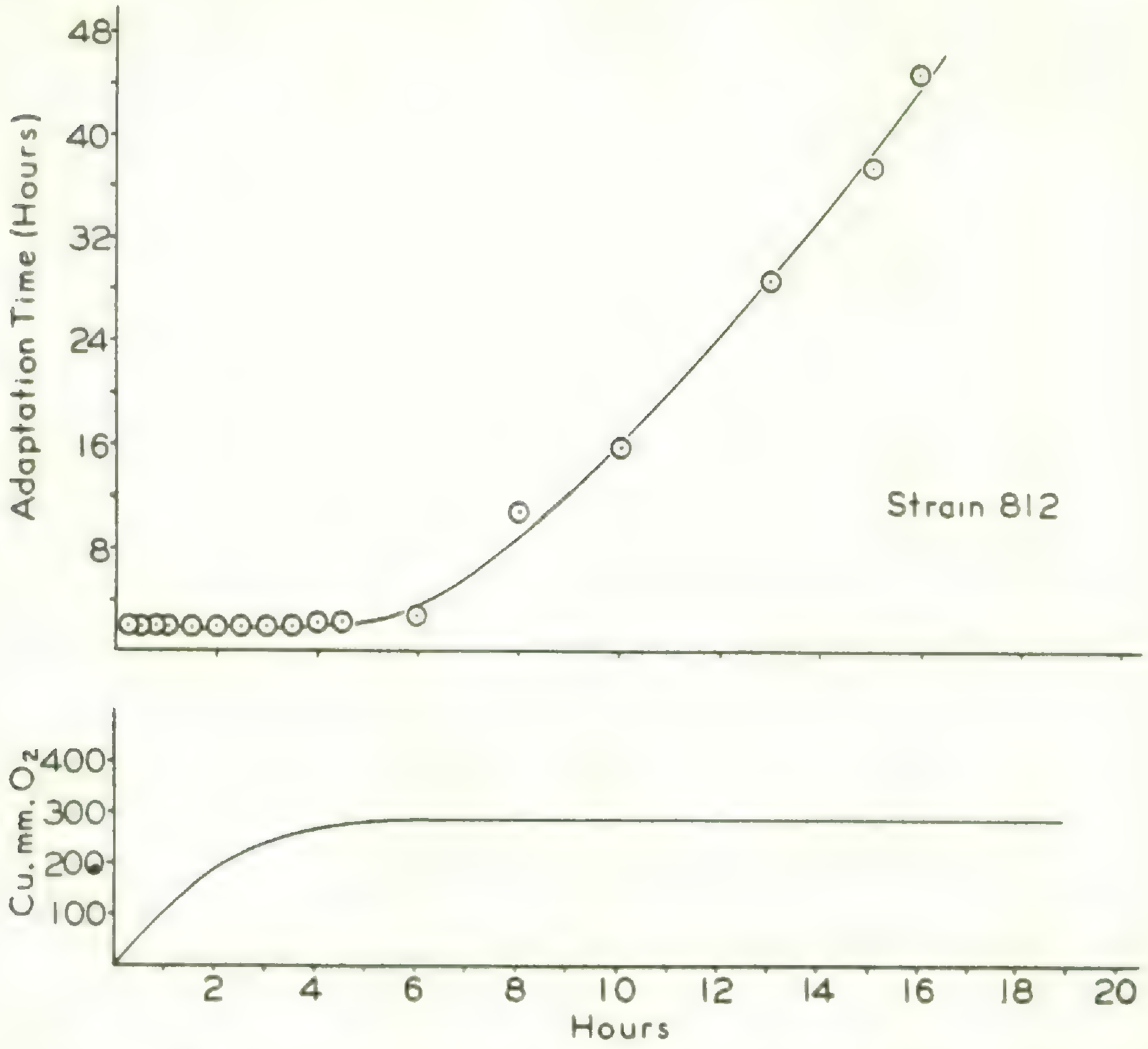


Fig. 3. The effect on adaptation time of adding the galactose at different levels of the endogenous respiration.

situation was, however, clarified by the finding (Spiegelman, '45b) that the galactose itself is oxidized by some enzyme system other than the fermentative one, which forms later under its stimulation.

It is clear from these experiments that the adaptation is intimately connected with the metabolic activity of the cells. Both aerobic and anaerobic processes are equally capable of supplying the energy for synthesis. Further experiments, which will be detailed elsewhere, indicate that agents which interfere with nitrogen assimilation (e. g., azide) completely suppress adaptation.

GENETIC INFERENCES FROM THE KINETICS OF ADAPTATIONS

It was pointed out in the introduction that, since enzymatic adaptations involved enzyme formation, a careful study of such processes could provide a clue as to the nature of the controls exercised by the genes over the enzyme constitution of cells. The most obvious and easily measured aspect of adaptation is the increase in enzymatic activity observed in non-dividing cells placed in contact with substrate. The usual description of gene action assumes that the gene

mediates directly the reproduction of the enzyme which it controls. From this point of view, every replication of every enzyme would require the intervention of the appropriate gene. On this basis we would ascribe the increase in enzyme activity, observed in the presence of substrate, to the stabilizing influence of substrate on the enzyme. It is proper to inquire what kind of activity time curve such an hypothesis would predict. We may picture the above mechanism by the following reaction diagram.[2]

$$\text{Gene} \downarrow \qquad \text{P} \underset{k'}{\overset{k}{\rightleftharpoons}} \text{E} \qquad (1)$$

Here *P* is the immediate precursor (perhaps some indifferent protein) whose transformation yields *E*, the enzyme, the activity of which is being measured. The velocity constant of the transformation from *P* to *E* is *k*, and its magnitude is determined by the gene controlling the reaction. The enzyme *E* is, however, very unstable and reverts to *P* quickly, the velocity constant of the back reaction being very much larger than that of the forward one. Under such conditions only very small amounts of *E* would accumulate in the cell. We now assume that substrate *S* stabilizes *E* and that in the presence of excess substrate, *ES* is formed predominantly. This effectively suppresses the value of *k'*. In the presence of substrate, the rate of the appearance of enzyme is then described by:

$$\frac{dE}{dt} = k(\bar{P} - E) \qquad (2)$$

where *E* represents the number of units of *P* transformed into enzyme in unit time and $\bar{P}$ is the initial amount of precursor present. Integrating and assuming for simplicity that *E* is zero when *t* is zero, we find:

$$E = \bar{P} - \bar{P}e^{-kt} \qquad (3)$$

According to equation (3), the assumption that the enzyme is increasing, due to synthesis by the gene and stabilization by substrate, predicts the curve depicted in fig. (4a) as the shape of the activity-time curve during the course of the adaptation. This curve implies that, during the entire period of adaptation, the rate of enzyme synthesis should decrease continuously at any given moment, in a manner proportional to the amount of new enzyme formed at that time. In the

[2] It may be noted that this system is a logical analogue of Yudkin's ('38) "mass action" theory of enzyme synthesis.

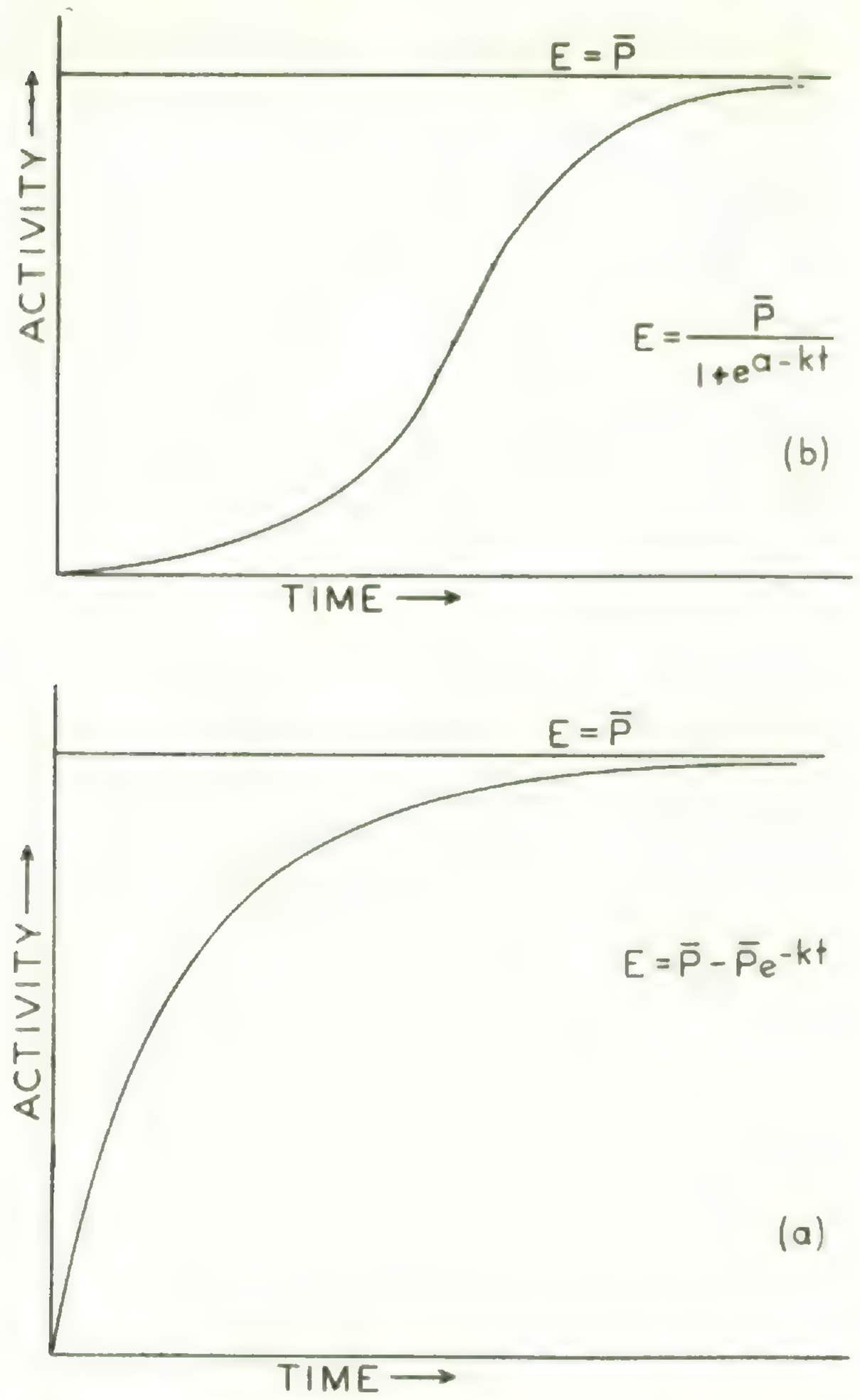
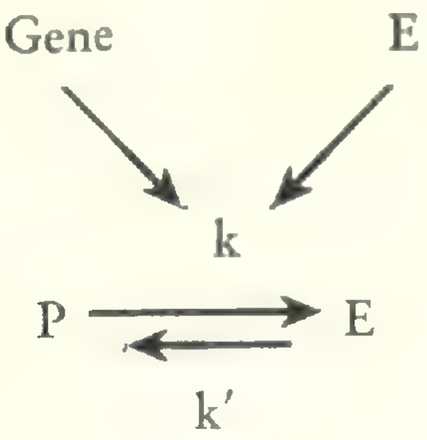


Fig. 4. Activity-time curves predicted by (a) direct primary gene control of enzyme synthesis and (b) self-duplication of enzyme molecules.

course of examining the synthesis of the glucozymase and melibiozymase systems, over 400 adaptation curves have been obtained. In no case does the activity-time curve resemble the course predicted by the above analysis. In all instances (see e. g. fig. 2, open circles) the initial part of the curve is characterized by a rising rate of enzyme formation. This is then followed by a declining rate portion, when presumably the indifferent substrate becomes limiting and finally exhausted.

The increasing rate of enzyme synthesis, with increasing amount of enzyme, suggested an obvious modification of the mechanism detailed in diagram (1). Retaining all the properties ascribed to the first mechanism, we add the additional one that the enzyme once formed can duplicate itself without further need for genic intervention. With this self-duplication hypothesis, instead of reaction diagram (1), we have,



(4)

where the symbols have the same meaning. As before, we suppose that the gene can transform *P* into active enzyme *E*, which is unstable in the absence of substrate. The arrow going from *E* to *k* symbolizes the self-duplication of the enzyme, which would express itself in terms of changing velocity constant so that its value at any particular moment would depend on the amount of *E* present. In the presence of substrate, the rate of formation of the enzyme becomes, under these assumptions, a quadratic function of the amount of enzyme present and takes the form:

$$\frac{dE}{dt} = kE(\bar{P} - E) \qquad (5)$$

where *E* again represents the amount of *P* transformed into *E* in unit time and $\bar{P}$ is the initial amount of precursor present. Integrating equation (5) we obtain

$$E = \frac{\bar{P}}{1 + e^{a - kt}} \qquad (6)$$

where *a* is an integration constant determined by initial conditions. According to equation (5) then, the assumption of self-duplication predicts the s-shaped curve given in fig. 4b as the activity-time curve during adaptation.

There is no doubt that the data lend support to the self-duplication hypothesis and rule out the simple genic mechanism underlying reaction diagram (1). More rigorous mathematical and experimental tests have been made and will be detailed elsewhere. An important and critical prediction stemming from the self-duplication mechanism is that, once the process is started, it can proceed in the absence of the gene which initiated it. Attempts to test this prediction were made with data on the inheritance of melibiozymase. The Mendelian mechanism underlying the inheritance of the ability to form melibiozymase was analyzed with the aid of two strains differing in this character.

THE GENETICS OF THE ABILITY TO FORM MELIBIOZYMASE

Hybrids between melibiose-fermenters and non-fermenters had already been examined by Winge and Laustsen ('39a). All hybrids of such crosses were fer-

menters, and their results led Winge and Laustsen to state that "the presence of a specific enzyme is dominant to its absence in all the instances studied." They made matings by placing two spores in contact with each other. This method has the disadvantage that one cannot characterize the haplophase parents, since both of the original spores are consumed in the mating. This, and the failure to examine the phenotypes of the segregants from the hybrids, prevented an analysis of the genetic mechanism.

This analysis was undertaken by Lindegren, Spiegelman and Lindegren ('44), using S. *carlsbergensis*, which could adapt to ferment melibiose, and S. *cerevisiae*, which could not. In this investigation hybrids were produced by mixing haplophase cultures. Since only part of the culture is needed for the mating, the remainder could be used to determine the characteristics of the parent strain, as well as for back-crossing or mating to other clones of interest. All hybrids formed were allowed to sporulate and the asci dissected to permit examination of the phenotypes of the haploid segregants.

The data obtained from 175 progenies of the interspecific and of related hybrids were consistent with the view that S. *carlsbergensis* contains two pairs of dominant genes (mel+), either one of which permitted the production of melibiozymase. Since all of the haploid segregants of S. *cerevisiae* failed to produce the enzyme, it was clear that it was homozygous for the recessive alleles.

SELF-DUPLICATION OF MELIBIOZYMASE IN THE ABSENCE OF ITS GENE

With the genetics of the capacity to form melibiozymase known, it became possible to devise experiments which would test the self-duplicating hypothesis suggested by the S-shaped adaptation curves. In particular, it was essential to provide answers to the following questions:

(1) If synthesis has been initiated and the gene's allele substituted by segregation, can the substrate-cytoplasmic interaction maintain the enzyme indefinitely in the cytoplasm in the absence of the specific gene?

(2) If some enzyme is present, can synthesis of additional enzyme occur in the absence of the specific gene necessary to initiate its synthesis?

Use was made of progenies of known genetic composition from the S. *cerevisiae* x S. *carlsbergensis* pedigree employed in the study of the Mendelian mechanism of melibiozymase inheritance. In the experiments described in the previous section, the cells came into contact with melibiose for the first time in the test for adaptability after segregation had already taken place. To answer the questions posed above, experiments were performed (Spiegelman, Lindegren and Lindegren, '45), in which the matings as well as the segregations were carried out in the presence of melibiose. The results were compared with matings from the same cross in which melibiose was omitted until testing the phenotype of the haploid segregants. To simplify the genetics of the situation, a haplophase clone carrying a single mel+ gene controlling adaptation was used. This was mated to a haplophase clone of S. *cerevisiae* which carried only the recessive alleles. The heterozy-

gous diploids so formed were all adaptable and each four-spored ascus from these hybrids yielded two adaptable and two unadaptable haplophase cultures.

TABLE II

EFFECT OF MELIBIOSE ON PHENOTYPIC CHARACTERS OF SEGREGANTS FROM DIPLOIDS FORMED BY MATINGS IN ITS PRESENCE AND ABSENCE

Mating, sporulation and planting in presence of melibiose					Mating, sporulation and planting in absence of melibiose				
Ascus No.	Spores* A	B	C	D	Ascus No.	Spores* A	B	C	D
1	+	+	+	+	8	+	+	−	−
2	+	+	+	+	9	−	+	−	+
3	+	+	+	+	10	+	+	−	−
4	+	+	+	+	11	+	+	−	−
5	+	+	+	+	12	−	+	−	+
6	+	+	+	+	13	+	−	−	+
7	+	+	−	−	14	+	+	−	−
					15	+	+	−	−
					16	+	+	−	−
					17	−	−	+	+

* + indicates ability to ferment melibiose, — inability. All spores come from a (+ x —) cross. See text for further details.

The data obtained on the phenotypes of the haploid segregants from diploids formed and segregated in the presence and absence of melibiose are summarized in Table II. Asci 1–15 originated from the mating of the same pair of mel+/mel— haploids, while 16 and 17 originated from mating an equivalent, but not identical, pair of mel+/mel— haploids. Melibiose was present during all stages of the formation and dissection of asci 1–7 inclusive. Asci 10–17 inclusive were formed in the usual way, without melibiose. In handling asci 8 and 9, the agar in which the dissected spores were planted contained melibiose.

It is evident from Table II that all asci formed in the complete absence of melibiose give the typical 1:1 ratio characteristic of a heterozygous hybrid segregating a single pair of genes. On the other hand, with the exception of ascus No. 7, identical heterozygotes treated with melibiose yielded four adaptable spores from each ascus.

The results obtained in the absence of melibiose prove that only 2 spores from each tetrad in asci 1–6 inclusive contain the specific mel+ gene responsible for

adaptation to fermentation. Despite this, all four spores from these tetrads produced haplophase cultures which fermented melibiose.

Since all steps were carried out in the presence of melibiose, selection of adaptable mutants from haploids originally unable to ferment melibiose might have occurred. Several specific facts, however, rule out this possibility: (1) During the testing of many haploid segregants from S. *cerevisiae*, all of which are negative, no mutation to an adaptable type has ever been observed whether melibiose was present or not; (2) the same is true of negative haploids from heterozygous hybrids. No mutation to adaptables in these have been seen no matter how often they have been through melibiose media; (3) asci 8 and 9, whose segregants were planted on melibiose, yielded the standard 1:1 ratio.

Presumably, the cultures from the two spores of each tetrad from the first six asci were able to ferment melibiose only due to the presence of the enzyme in the cytoplasm. On this basis it was to be expected that removal of the melibiose would lead not only to the disappearance of fermentability in all cases, but to an eventual loss of readaptability in two of every four cultures arising from each of the first six asci. To exclude the complication of mutation away from adaptability, non-dividing cultures, suspended in M/15 KH_2PO_4, were used. Portions of all 24 adapted haplophase cultures originating from the first 6 asci were dissimilated in the absence of substrate until they had lost all melibiozymase activity. Samples were then removed and incubated with melibiose to test for readaptability. Not all haplophase cultures survived this relatively vigorous treatment which in some cases lasted 20 days. Table III summarizes the results obtained with those asci, all four of whose segregants stood the treatment. The removal of the melibiose and its stabilizing influence leads to the disappearance of the enzyme in the cytoplasm and the reappearance of the expected Mendelian ratios.

Data collected at the same time indicate that synthesis of additional enzyme can occur in the absence of the mel+ gene. After allowing all suspensions to

TABLE III

READAPTABILITY OF SPORES OBTAINED BY MATINGS IN PRESENCE OF MELIBIOSE AFTER HAVING LOST ALL ADAPTIVE ENZYMES

Ascus No.	Spores* A	B	C	D
1	+	−	+	−
2	−	−	+	+
4	+	+	−	−
6	+	+	−	−

* + indicates readaptability, − inability.

fall to low $Q^{N}_{CO_2}$ values (between 1.8 and 10.1) in the absence of melibiose, portions were removed and incubated with melibiose and regeneration of activity followed at intervals by measuring $Q^{N}_{CO_2}$. The results of those haploid segregants which subsequently lost the ability to adapt are recorded in Table IV. It is seen that in all cases marked increases in activity were obtained. Furthermore, all the strains listed in Table IV were carried in standard media with melibiose and were tested at weekly intervals. At the end of three months they could all ferment melibiose at rates equal to, or greater than, the original rate. This period is equivalent to over 2,000 cell generations. It is evident that the enzyme can not only maintain itself in the presence of melibiose but it can also increase in absolute amount.

TABLE IV

$Q^{N}_{CO_2}$ VALUES AFTER AEROBIC INCUBATION WITH MELIBIOSE OF STRAINS WHICH EVENTUALLY LOST THEIR ABILITY TO ADAPT

Strain	Hours of contact with melibiose			
	0	12	24	48
1B	5.1	40	96	123
1D	2.4	26	109	114
2A	10.1	39	86	136
2B	6.3	46	73	101
4C	5.0	69	160	170
4D	4.2	29	91	134
6C	1.8	34	84	141
6D	4.8	42	121	130

The simplest explanation which can be offered at present for the above results is the same one advanced for the S-shaped curve, i. e., the effect of substrate on a self-duplicating enzyme. We may thus explain the effects of melibiose on the inheritance of melibiozymase as follows: by performing the mating in the presence of melibiose, the cytoplasm of the haploid carrying the mel+ gene is packed with the melibiose-fermenting enzyme. Since both copulating haploids contribute cytoplasm equally to the zygote it starts out with some enzyme and builds up more since it has the gene also. Since sporulation occurs in the presence of melibiose and since the sporulation period is characterized by growth and considerable storage, the enzyme molecules are stabilized and possibly increased in amount. Each of the four haploid segregants derives its cytoplasm from the diploid hybrid, and it follows that each will have enzyme molecules in its cytoplasm no matter

what its genetic constitution. Finally, the enzyme molecules are stabilized and duplicate themselves in the descendants of the spores which do not have the mel+ gene as long as they are kept in contact with the substrate.

It cannot be denied that explanations of these results involving unstable genes can be devised. It is further recognized that no analysis of the adaptation curves, no matter how rigorously it is formulated and subsequently tested, can ever prove the self-duplication of enzymes. It is impossible to exclude in any finite period all the conceivable modifications which can be advanced containing as their primary postulate the gene as the sole self-duplicating unit in cells. This much, however, may be said in view of the experiments on melibiozymase and galactozymase—such theorizations will not be "pleasingly simple."

We may, therefore, on the basis of the available evidence, suggest that some enzymes are capable of duplicating themselves without genic intervention. In these cases of enzyme formation, the sole function assignable to the gene is the initiation of the enzyme synthesis. This initiation could be effected by virtue of a low but ever-present capacity of the gene to mediate the production of a few enzyme molecules. A mechanism of this kind would keep some enzyme molecules always available in the cytoplasm for autosynthetic activity when substrate is supplied. It would, at the same time, explain the observed Mendelian inheritance of adaptability in the absence of substrate, as well as the ability of substrate when present to obscure the Mendelian ratios during segregation. In addition, it would provide a rational basis for the S-shaped adaptive curves.

The question of how generally the above concept can be applied cannot be decided without further experiments on other enzyme systems. From a casual observation it might seem that self-duplication of the enzymes is inconsistent with the results on dosage effects. It must, however, be noted that in such studies, end products of enzyme activity, rather than enzymes themselves, are being studied. Furthermore, in order for the self-replication capacity of enzymes to express itself, the precursors would have to be initially present in sufficient quantities. If the enzyme precursor is limiting, the effect of E on the forward velocity constants would be negligible, and diagram (4) would transform to diagram (1). Then, even if the enzyme was able to duplicate itself, the synthesis would be predominantly gene-controlled. The addition of another gene would, under such conditions, effect the quantitative level of enzyme activity. To this must be added, in so far as gene-dosage studies are concerned, possible limitations of the precursor to the end product. In this connection, it is of interest to note that competitive interaction for a limited amount of substrate has been assumed in Dr. Stern's theorizations on the nature of gene action.

Finally, it must be noted, from a general physiological point of view, that self-duplication of enzymes provides a degree of physiological flexibility not easily attained with the older, more rigid concepts of gene control over enzymatic constitution. Under this concept the enzymatic composition is dependent not only upon the genome, but also on the available substrates. Enzymes could thus

increase and decrease in response to the substances placed in their environment. In addition, it provides an experimentally interesting mechanism for cytoplasmic differentiation, since it predicts that cells with the identical genomes need not possess identical enzymatic constitutions.

SUMMARY

The general problem of enzymatic adaptation is discussed. The view is adopted that adaptive-enzyme formation is a quantitatively exaggerated instance of a more general phenomenon involving the effects of substrates on the synthesis and stability of their enzymes. Experiments on the genetic control of adaptation to the fermentation of galactose and melibiose by yeasts are described and discussed in detail. It is pointed out that these instances, where enzyme formation can be followed with relative ease, offer a unique opportunity for examining the often-repeated concept that genes determine phenotype by virtue of their control of the enzymatic constitution of cells. In particular, the extent and mechanism of such control might thus be open to experimental analysis.

A study of the kinetics of the formation of galactozymase and melibiozymase in yeast cells is detailed which suggested that at least in these cases the enzymes were capable of self-duplication without the necessity of genic intervention. The hypothesis of self-duplication led to the prediction that such enzymes, once formed, should maintain themselves and be transferable from one cell generation to the next in the complete absence of their corresponding genes. Experiments on the inheritance of melibiozymase in the presence and absence of melibiose are reported which tend to confirm this prediction. It is suggested that, in these cases of enzyme formation, the sole function assignable to the gene is the initiation of the enzyme synthesis, this initiation being effected by virtue of a low but ever-present capacity of the gene to mediate the reproduction of a few enzyme molecules. Subsequent replication is dominated by the self-duplicating enzyme molecules.

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THE MECHANISM OF RADIATION EFFECTS AND THE USE OF RADIATION FOR THE PRODUCTION OF MUTATIONS WITH IMPROVED FERMENTATION

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PART I

In discussions of the effects of radiation, the ultraviolet spectrum is usually divided into the so-called biologically effective region between 2000 and 3200Å and the non-active region longer than 3300Å. Studies conducted during the last few years have shown that both of these regions of the spectrum are effective. However, the energies necessary to produce recognizable effects are of a different order of magnitude. The modes of action of the various wavelengths of the ultraviolet are fundamentally different, apparently affecting various structures of the cell.

The region shorter than 3200Å is characterized by its high absorption by proteins and nucleic acids, the proteins by their low absorption band in the 2800Å region and high absorption at wavelengths shorter than 2300Å; the nucleic acids by their extremely high absorption band at 2600Å. In general, absorption spectra of biological material will show a pattern resembling protein absorption or show slight modification usually indicating nucleoproteins. It is only when the nucleic acid is concentrated in certain structures as, for instance, chromosomes, that its location can be readily recognized as has been shown by Caspersson ('36).

Considerable information in regard to the chemical characterization of the biological effect of radiation can be obtained from wavelength dependence studies of biological effects. Another method for determining what radiation will do to the cell is to extract its chemical constituents and follow their change *in vitro* by certain physical and chemical techniques. A further method is to follow changes in certain morphological structures produced by specific wavelengths in living cells. We have used all three approaches in our studies. However, we have obtained the most extensive data by the first method which I have mentioned, and rather fragmentary data by the other approaches. In studying the effects of radiation on biological materials, we have concentrated our efforts on problems which would be of direct or indirect significance to public health. This, of course, is not very difficult, since any fundamental biological approach will help us with the interpretation of the relation of disease to health.

I will discuss first a typical wavelength dependence study which we have recently completed (Hollaender and Oliphant, '44). The sensitivity of influenza virus A was determined for 8 wavelengths in the ultraviolet spectrum between 2180 and 2967Å. To get a definite measure of the sensitivity of this virus, we have irradiated a standard culture of *Escherichia coli* in the virus suspension. The

sensitivity of this organism to monochromatic radiation is well established. We found only a slight difference in the resistance of *Escherichia coli* and influenza virus. There is also very little difference in wavelength dependence of their inactivation. The absorption spectrum of influenza virus, as well as of bacteria, shows predominantly the type that you would expect from proteins mixed with a small percentage of nucleoproteins, whereas the inactivation spectra resemble more closely the pure nucleic acid absorption spectrum.

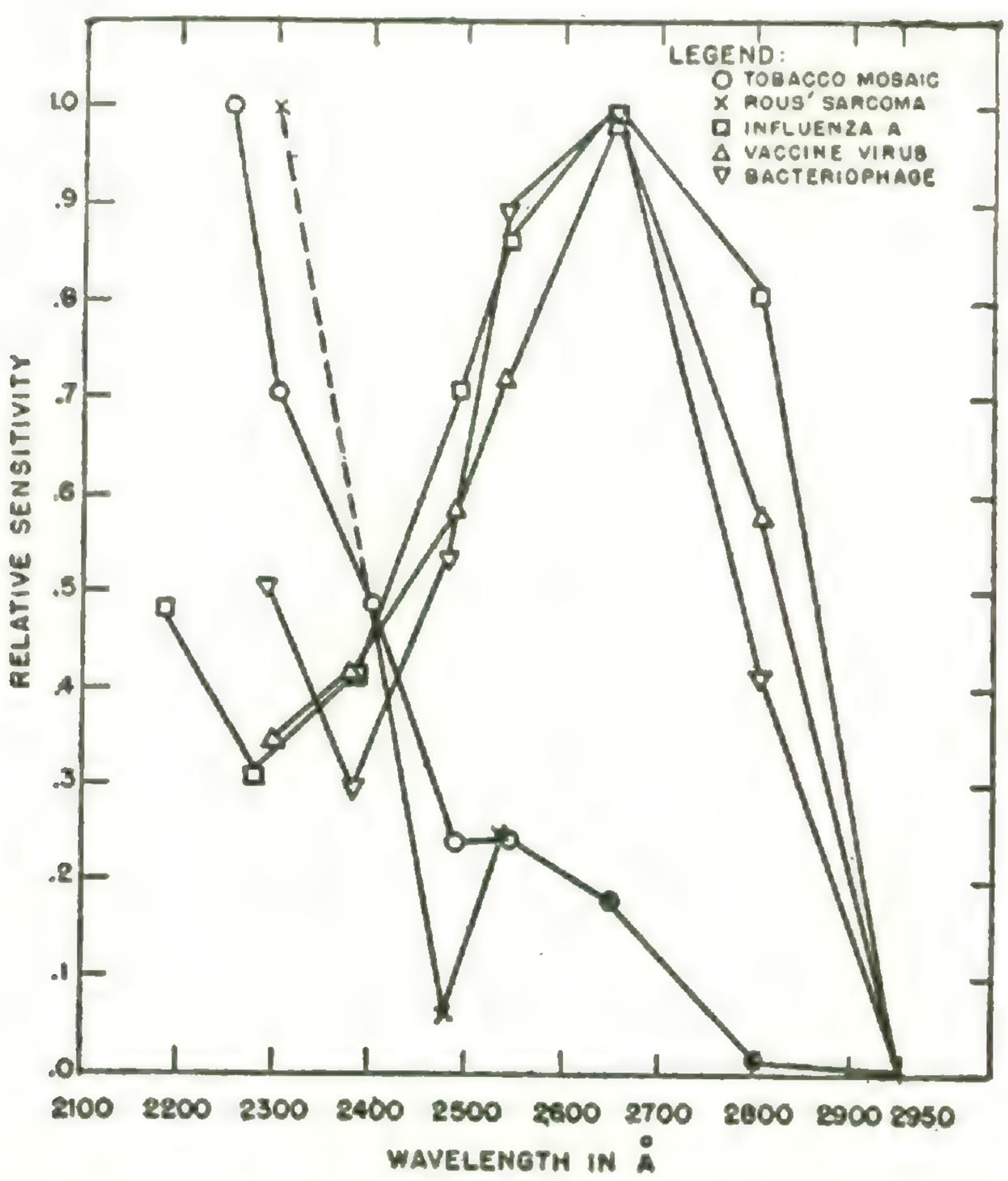

Fig. 1. Plot of the relative sensitivity against the wavelength for tobacco mosaic, Rous' sarcoma, influenza A, vaccine virus and bacteriophage, taking the energy at the wavelength which is most effective as 1 and dividing by the less effective energies. (For references see Table I, Hollaender and Oliphant, '44.)

Do all the viruses behave the same way? There are available activity spectra for five viruses: three of these have a maximum of sensitivity at 2650Å and decreasing sensitivity at shorter or longer wavelengths, and two which have a high sensitivity at 2300Å and considerably lower sensitivity in the 2600Å range. Influenza A, vaccine virus, and bacteriophage belong to the first group, and the viruses of typical tobacco mosaic and of Rous' sarcoma belong to the latter group. I mentioned before that the absorption spectra of viruses resemble more closely protein spectra with a small band typical for nucleic acids. There seems to be little doubt that, on the basis of these findings, the nucleic-acid part of the influenza, vaccine virus, and bacteriophage is the most sensitive part of these

particles, and that the protein part of tobacco mosaic and Rous' sarcoma virus is the least resistant one.

Another point which should be emphasized is that the nucleic acid of influenza, vaccine virus, and bacteriophage has been reported to be desoxypentose and the nucleic acid of tobacco mosaic and Rous' sarcoma is predominantly pentose. (For details of this study see Hollaender and Oliphant, '44. See later discussion of irradiation of nucleic acids *in vitro*.)

TABLE I

REACTIONS WITH HIGHEST SENSITIVITY AT 2650Å

Inactivation of viruses and virus-like agents		Influenza (Hollaender & Oliphant, '44) Vaccine (Rivers & Gates, '28) Bacteriophage (*St. aureus*) (Gates, '34)
Killing of microorganisms	Bacteria	All types—Pathogenic and Saprophytes — For review see Hollaender, '42
	Fungi	Yeasts (Oster, '34) *Trichophyton* (Hollaender & Emmons, '39) *Aspergillus terreus* (Hollaender, Raper & Coghill, '45)
Mutation production	Fungi	*Trichophyton* (Emmons & Hollaender, '39) *Neurospora* (Hollaender, Sansome, Zimmer & Demerec, '45) *Aspergillus terreus* (Raper, Coghill & Hollaender, '45) *Penicillium notatum* (Hollaender & Zimmer, '45)
	Higher Organisms	*Sphaerocarpus* (Knapp, Reuss, Risse & Schreiber, '39) *Zea Mays* (Stadler & Uber, '42) *Drosophila* — MacKenzie & Muller, '40; Demerec, Hollaender, Houlahan & Bishop, '42
REACTIONS WITH VERY HIGH SENSITIVITY AT λ 2300Å AND A SMALL MAXIMUM AT 2800Å OR 2600Å		
Inactivation of viruses and enzymes		Tobacco mosaic (Hollaender & Duggar, '36) Rous' sarcoma (Sturm, Gates & Murphy, '32) Urease (Kubowitz & Haas, '33)
Killing of higher organisms		*Enterobius vermicularis* (Hollaender, Jones & Jacobs, '40) *Ascaris* (Wright & MacAlister, '34)
Parthenogenesis of *Arbacia*		*Arbacia* (Hollaender, '38)

Table I shows a list of biological reactions for which sufficient data are available on the effect of monochromatic ultraviolet radiation to permit their being

classified and fitted into the predominantly protein or the nucleic acid pattern. It is not surprising that bacteria have a maximum sensitivity at 2650Å, since, as far as tested, they are made up of a high percentage of nucleic acids. The same applies to yeasts and fungi but the 2600Å maximum of sensitivity in many fungi is obscured by the protective absorption of pigments.

Whenever 2600Å radiation has been tested for mutation production and the conditions have been such that the radiation could penetrate readily to the nucleus, high efficiency in producing genetical changes for this wavelength has been found. Our early studies with Dr. Emmons on *Trichophyton* have now been repeated with *Neurospora* in a cooperative study with Dr. Demerec and Mrs. Sansome. The results of this study verify our findings on *Trichophyton:* 2650Å is the most efficient wavelength in producing mutations. This work on fungi, which I will discuss in the second part of my paper, has also been extended to *Penicillium notatum* and *Aspergillus terreus.*

I am sure you are acquainted with the work on higher organisms. I included the work on *Drosophila* under the 2650Å section in spite of the fact that the wavelength which is most effective on this organism is 3130Å. The probable reason for this is that the sperm has to be irradiated inside the fly and the abdominal wall prevents the 2650 wavelength from penetrating readily to the sperm. It is unfortunate that the artificial insemination technique has not proved practical.

The second part of this table shows a number of biological reactions with high sensitivity in the very short ultraviolet (<2300Å). This would indicate that the protein part of these materials is the most sensitive one. The inactivation of tobacco mosaic and Rous' sarcoma shows also a small maximum at λ 2600Å, indicating that the nucleic-acid part of these materials has a slight sensitivity in this region.

The higher organisms described in the rest of this table are surrounded by heavy protein membranes which explain their sensitivity in the short ultraviolet. Little information is available at the present time in regard to tissue cultures. Crude work has shown that this material shows its highest sensitivity at short wavelengths.

It would not be surprising, however, that careful studies which take into account the action of protective materials would bring out a fairly high sensitivity in the 2600Å region.

Summarizing, it is well to point out that the wavelength-dependence studies have given us an opportunity to obtain an indication of the chemical structure in living substance, which is most easily interfered with by radiation.

In an effort to get a better understanding of the effect of radiation on living materials, we studied some of the constituents of living cells *in vitro.* We have studied the effect of 2537Å radiation on sodium thymonucleate (Hollaender, Greenstein and Jenrette, '41) and certain serum proteins (Davis, Hollaender and Greenstein, '41). The changes most readily produced are the result of alteration in the

physical properties of the treated compounds, for example: viscosity, stream birefringence and colloid osmotic pressure. While the changes produced in the isolated compounds of the living cell or directly in the living cell are doubtless qualitatively similar, quantitatively they must appear to differ enormously. This is probably due to the difference in detectability of the two types. Changes in the isolated components must be detected by physical methods which require that a relatively large number of the molecules of the compounds under study be altered, and this, in turn, requires very large doses of radiation. The structures of the living cell, on the other hand, even though they consist of these same or similar compounds are parts of very delicately balanced and precisely adjusted units, in which changes induced in a few molecules by relatively low doses of radiation may alter radically certain detectable behavior and structural characteristics of the cell (Carlson and Hollaender, '44). Very little is known about the state of the relation of protein to nucleic acids in living cells. A search of the literature on this subject reveals that there is still considerable confusion about the exact structure of nucleo proteins (Greenstein, '44) and further work in this field is urgently needed.

The effect of 2537Å was studied (Carlson and Hollaender, '44; Kaufman, Gay and Hollaender, '44) in an effort to obtain information in regard to the mechanism of the influence of radiation on mitosis. Although this study is in its early phases, the results indicate that the early prophase is retarded most by 2537Å radiation, in contrast to X-rays where the middle and late prophases are most sensitive. The high sensitivity of chromosomes to 2537Å radiation is well demonstrated by the fact that an exposure to a total of 1500 ergs per square centimeter, either given in 1 second or spread over 1500 seconds, will produce a measurable retardation of mitosis in grasshopper neuroblasts in tissue cultures.

Up to this point, I have discussed the effects of radiation shorter than 3200Å. The action of radiation in the long ultraviolet has been more or less ignored. One reason is that most non-pigmented biological materials have very little absorption in this region; as a matter of fact, so little that our present means of taking absorption spectra are not sensitive enough to detect this absorption. This also explains why the energies necessary to produce changes in the long ultraviolet are of different order of magnitude than the ones at shorter wavelengths. For instance, we can produce very striking effects if we give bacteria 10,000 to 100,000 as much energy at 3650Å as was necessary to produce recognizable effect at 2650Å. Besides its lethal action, 2650Å will produce a delay of growth in surviving organisms; in other words, a prolongation of the "lag" phases. This prolongation of the lag will be about 50 per cent of the normal lag. The 3650Å range may increase the normal lag phase tenfold. It will also change the permeability of the cell. We have summarized these effects in Table II.

It appears that the effect produced by this wavelength is through action on the colloid structure of materials irradiated as well as in the structure of certain respiratory enzymes. The function of the long ultraviolet is important from

TABLE II

EFFECTS OF LONG ULTRAVIOLET AND NEAR VISIBLE RADIATION ON *ESCHERICHIA COLI*

	3400 to 4400Å	2180 to 2967Å
1. Shape of killing curve (log survival ratio/energy)	Threshold type	Approaching straight line
2. Energy (incident) for 50% survival ratio	Approximately 2×10^8 ergs/cm.^{2}	5×10^2 to 10^3 ergs/cm.^{2}
3. Temperature coefficient	1.7 — 2.2	1.1
4. Sublethal effects appear	Before any organisms are killed (in threshold part of killing curve)	After 60 to 90% of organisms are killed
5. Extension of retarded growth phase for 10% survival ratio	Up to 1000%	50%
6. Toxicity of certain salt solutions can be recognized	At once after irradiation	In 600 minutes at 32° C.
7. Mutation production	No mutations	Mutations produced in fungi and *Drosophila*

the ecological point of view, since this radiation is quite intense in sunlight.

In summary, the study of the response of microorganisms to ultraviolet radiation has established distinct effects which each wavelength range produces. The wavelengths which are most highly absorbed by nucleic acids (2600Å) are most efficient in producing mutations. Other wavelengths which are absorbed more generally by the cell (3650Å) show their effect in a retardation of growth and an interference with the normal respiration of the cell. Several regions of the spectrum still await an interpretation of their effects on the living cells. The field of the combination of different wavelength ranges is an especially promising one for further investigation.

PART II

The production of mutations by ultraviolet radiation follows a definite quantitative pattern. The maximum mutation rate is reached after the organisms have been exposed to certain amounts of energy. A further increase in energy tends to decrease the mutation rate from this maximum rate. In contrast to this, the increase of mutation rate with increasing energy in the X-ray region is more or less linear. These typical mutation curves have been established not only with the *Fungi Imperfecti* but have also been found with *Neurospora crassa* (Sansome, Demerec and Hollaender, '45; Hollaender, Sansome, Zimmer and Demerec, '45).

It was thought when this work was begun that it might be possible, by radiation techniques alone, to produce mutations of certain predetermined properties. Experience has shown that it is not yet possible to accomplish this. However, it has been found that the ultraviolet will produce a predominance of gene mutations while the X-rays tend to produce a predominance of chromosomal aberrations and chromosome breaks (Stadler and Uber, '42).

Most of the early work on mutation production in fungi established the mutation rate on the basis of "morphological" changes. But the fundamental reactions which cause the appearance of morphological mutations are no doubt "biochemical." Early in the war it became desirable to produce changes in certain organisms which were capable of producing urgently needed chemicals. This led us to suggest the use of radiation techniques for this purpose.

The usual tendency in all induced mutation work is to produce changes in the organisms which result in reduced activity. This is probably due to an interference with certain enzyme systems. This type of approach has been established by Beadle and Tatum ('41). The so-called "progressive mutations," i. e., mutations with improved fermentation, have only occurred occasionally. The difficulty here probably lies in the fact that several gene modifications are necessary to induce a mutation with increased yield while a "deficient" mutation may be caused by single gene changes.

The results of most fermentation processes of fungi are not alcohols, acids, etc., of high purity, but usually a mixture of more or less closely related compounds. Thus the suppression of an undesirable reaction, through interference with the enzyme system causing it, is a promising possibility. However, as can be seen below, if one interferes with one enzyme system, there is a tendency for the whole chain of systems to be disrupted, probably because of the close interrelationship of the different systems within the organism.

We will now discuss a number of studies where an attempt has been made to influence fermentation in a direction most desirable to the experimenter. Cultures which survived X-radiation and show deficiencies in development were observed as early as 1904 at a time when the early biological exploration of Roentgen's discovery was at its height (Dauphin, '04). Observations of Nadson ('25) showed that colonies of yeast on the border of irradiated areas in Petri dishes grow more profusely than the colonies protected against radiation.

An extensive study of the production of citric acid by *Aspergillus niger* as influenced by radium emanation and ultraviolet light was reported by Kresling and Stern in 1936. They observed an increase of citric acid in the cultures when grown in the presence of radon, but no increase of citric acid was observed when the cultures were grown under ultraviolet. A number of strains were isolated from the irradiated cultures. The results of these tests for acid production of these mutations are given in Table III. All of the new strains produce equal or less amounts of acid than the controls.

Early in the development of the mass production of penicillin by *Penicillium*

TABLE III

BIOCHEMICAL PROPERTIES OF RADIUM STRAINS OF *ASPERGILLUS NIGER**

Strains	Acid in grams per 100 ml fermentation solution			Sugar in grams per 100 ml solution		Dry weight of mycelium in grams
	Citric Acid	Gluconic Acid	Oxalic Acid	Used	Left over	
Control Strain #3	5.21	0.90	0.19	13.35	6.65	4.579
Radium Strain #3_1	0.18	2.91	0.00	11.66	8.34	3.822
Acid Strain #3_2	0.00	2.60	0.00	12.50	7.50	4.132
Control Strain #1	1.64	0.34	0.33	18.7	1.3	4.934
Radium Strain #1_1	0.00	0.58	0.00	15.0	5.0	4.872
Control Strain #6	11.88	1.16	0.33	18.7	1.3	3.554
Radium Strain #6_1	0.68	0.84	0.33	15.0	5.0	3.869
Radium Strain #6_2	0.00	2.33	0.20	14.0	6.0	4.761
Radium Strain #6_3	7.52	0.73	1.00	15.4	4.6	3.624

*Table taken from: Über die Wirkung von Radium- und ultravioletten Strahlen auf die Entwicklung, die biochemischen Eigenschaften und die Rassenbildung des *Aspergillus niger*. (Kresling and Stern, '36, p. 339).

TABLE IV

EXPERIMENT F3—*PENICILLIUM NOTATUM*. CULTURE 10 DAYS OLD IRRADIATED WITH λ 2650Å

	Energy per spore	Per cent survival	Number colonies isolated	Per cent mutation
Control	0	100	75	0
Run 1	3.0×10^{-3} ergs	43.0	74	1.3
2	9.4	37.4	76	1.3
3	19.5	14.7	75	2.0
4	34.1	5.4	81	13.6
5	42.9	.2	81	12.3
6	71.5	.03	80	8.8

notatum, we had an opportunity to discuss with Dr. Heatley of the Oxford group the possibility of producing mutations with increased penicillin yield by irradiating *Penicillium notatum* spores with monochromatic ultraviolet and possibly X-rays. We started this investigation in cooperation with Dr. Emmons of the National Institute of Health in 1941 and later continued it in cooperation with the Cold Spring Harbor group.

The production of the morphological mutations follows the usual pattern: high efficiency of mutation production with wavelength 2650, lower efficiency at shorter and longer wavelengths, and no mutation production at wavelengths in the 3650Å region. The mutation rate increased with increasing energy and became more or less erratic at still higher energy values. In contrast to this, the effect of X-rays on morphological mutations follows a more or less straight-line relationship. I will return to this point later on. Typical results of a single irradiation test are given in Table IV. A typical killing and a mutation curve are shown in fig. 2.

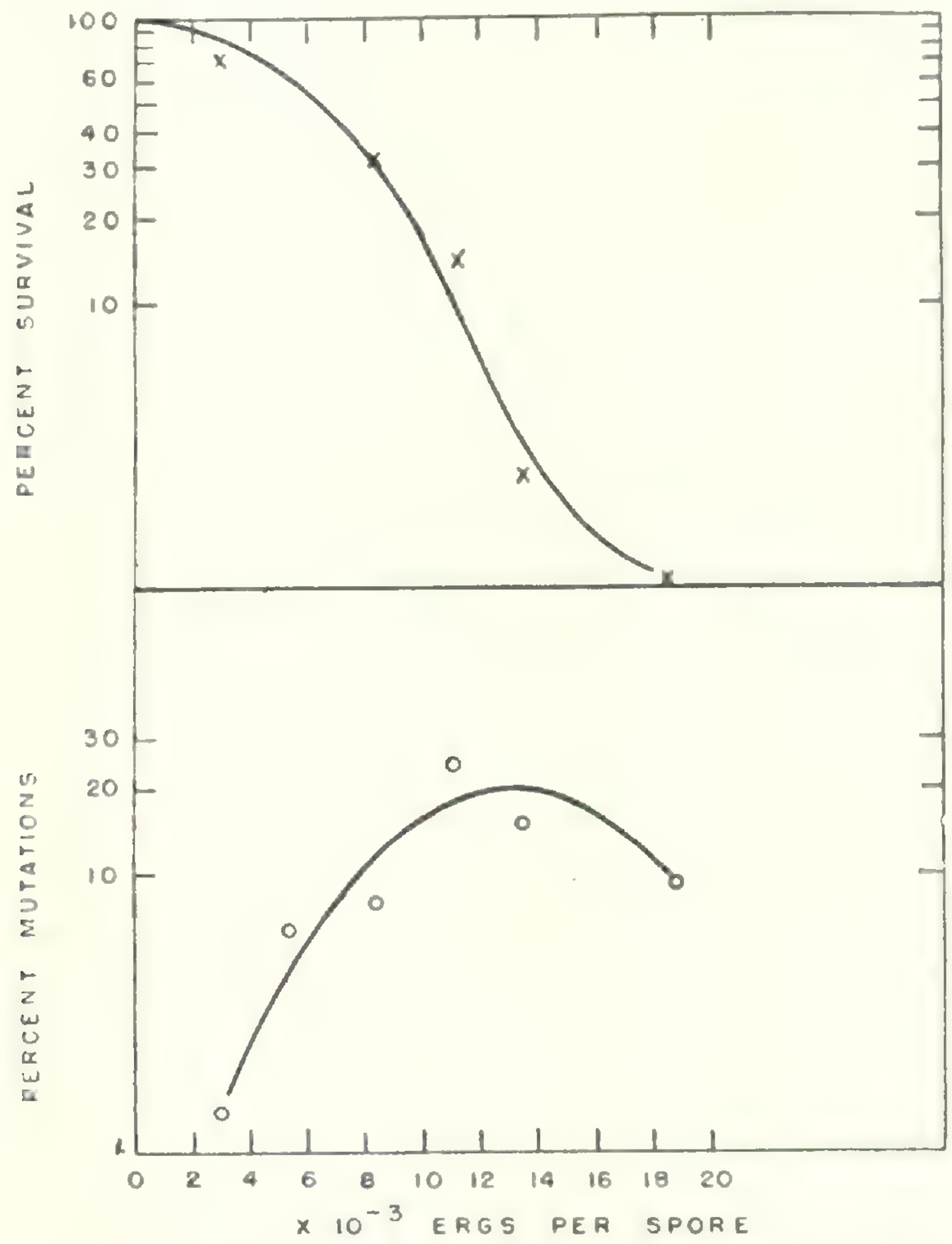

Fig. 2. *Upper graph:* Per cent survival against energy absorbed per spore for *Penicillium notatum*. *Lower graph:* Per cent mutation against energy. Each point on the lower graph corresponds to point in upper graph for same energy value.

No general biochemical investigation was conducted in connection with this study. However, in a collaborative study with Dr. J. W. Foster of Merck & Co.,

at Rahway, N. J., a number of irradiated and control cultures were tested for penicillin production at the Rahway laboratory. Penicillin production was determined after 2, 3, 4, and 5 days in submerged shaking cultures. The concentration was determined by the standard cup method. The results of a typical set of data are given in Table V. Typical yield distribution plots are given in fig. 3. The mean of the penicillin production for control and irradiated cultures is also given.

TABLE V

TYPICAL SET OF TESTS FOR CULTURES OF *PENICILLIUM NOTATUM* COMING FROM IRRADIATED AND CONTROL SPORES (JULY 1943)*

Culture	Oxford Units per ml		
	2 days	3 days	4 days
F_8 5.25	17	25	8
26	23	29	11
27	18	40	13
28	17	43	28
29	24	52	29
31	26	42	28
32	19	40	29
33	< 8	< 8	29
36	< 8	< 8	28
F_8 5.10	< 8	< 8	8
13	< 8	< 8	8
32	< 8	11	13
36	< 8	54	8
37	< 8	22	8
44	22	80	8
45	28	49	11
49	23	54	8
56	16	56	12
60	25	59	20
66	24	80	32
Control	33	46	17

*Tested by J. W. Foster, Merck & Co., Rahway, N. J.

The irradiated cultures show, in general, a very wide distribution of variation in the yield of penicillin with a predominance of low-yielding strains and some which practically did not produce any penicillin. However, occasionally a mutation was produced which gave an unusually high yield. Of about 200 cultures tested, two were found of this type. The distribution of yield of cultures seems to be definitely towards the lower side. It is unfortunate that the difficulty of testing *Penicillium notatum* for penicillin production makes it cumbersome to run through a large number of cultures under a variety of conditions which might bring out more clearly the interesting mutations. There seems to be little, if any, relation between morphological mutation and change in penicillin production. As a matter of fact, the normal-appearing cultures have a tendency to give the higher yields.

Another study was conducted in cooperation with Dr. Raper, Dr. Coghill and

others of the Northern Regional Laboratory (Hollaender, Raper and Coghill, '45; Raper, Coghill and Hollaender, '45). The purpose of this investigation was to attempt to produce mutations in *Aspergillus terreus* which would have increased itaconic acid production.

This organism distinguished itself by a very low sensitivity to ultraviolet radiation. However, it showed itself to be more sensitive to mutation production than any of the other organisms tested. While most of the organisms tested show their highest mutation rates with ultraviolet when 90 to 95 per cent of spores are killed, *Aspergillus terreus* shows its highest mutation rate after 25 to 40 per cent of the spores are inactivated.

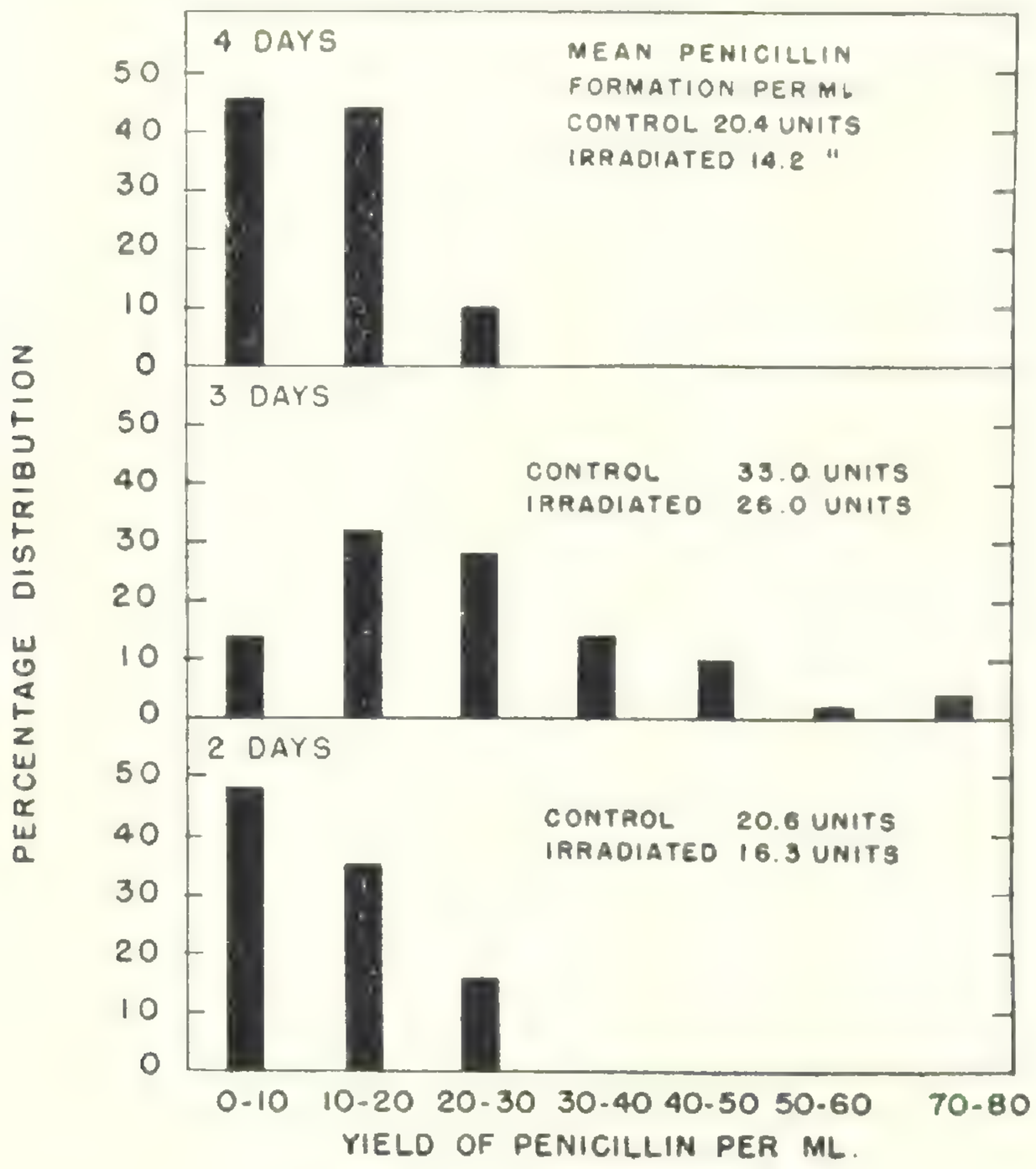

Fig. 3. Per cent distribution against yield of penicillin in Oxford Units based on tests made by Dr. J. W. Foster. Time of tests refers to days of incubation.

The morphological mutations showed wide variety in appearance. Several interesting mutations were found which showed certain deficiencies when grown in Czapek solution agar but which appeared normal on a more complete medium (malt extract agar). One of these is a thiamin-deficient mutation. When grown in Czapek solution agar it forms a thin spreading mycelium, while in malt extract agar it duplicates the normal mycelium. Another mutation appears deficient when grown on a nitrate medium, but when it is grown on a medium with ammonia or amino nitrogen the culture appears normal. A number of other deficiencies have appeared which await analysis.

In a separate study Lockwood, Raper, Moyer and Coghill ('45) investigated 217 irradiated cultures for their ability to produce itaconic acid. It was thought that it would be possible to inhibit some of the enzyme systems which would then leave the organism to ferment a higher percentage of the sugar to itaconic acid.

I am quoting from their summary:

"Nine different types of biochemical and cultural response have been observed from 217 strains of *Aspergillus terreus* derived from irradiated conidia.

"Among the 76 strains which were morphologically unchanged were 59 which appeared to be unaltered biochemically, 13 which produced more itaconic acid than the parent strain, and 4 which produced no itaconic acid.

"Among the 141 strains which were obviously altered morphologically were 42 strains not apparently altered biochemically, 88 which produced little acid, and 11 which did not grow on the test medium. None of these 141 strains produced more itaconic acid than did the parent strain.

"Fifteen strains produced considerable non-acidic unsaturated material.

"Seventeen strains appeared to produce no acid other than itaconic."

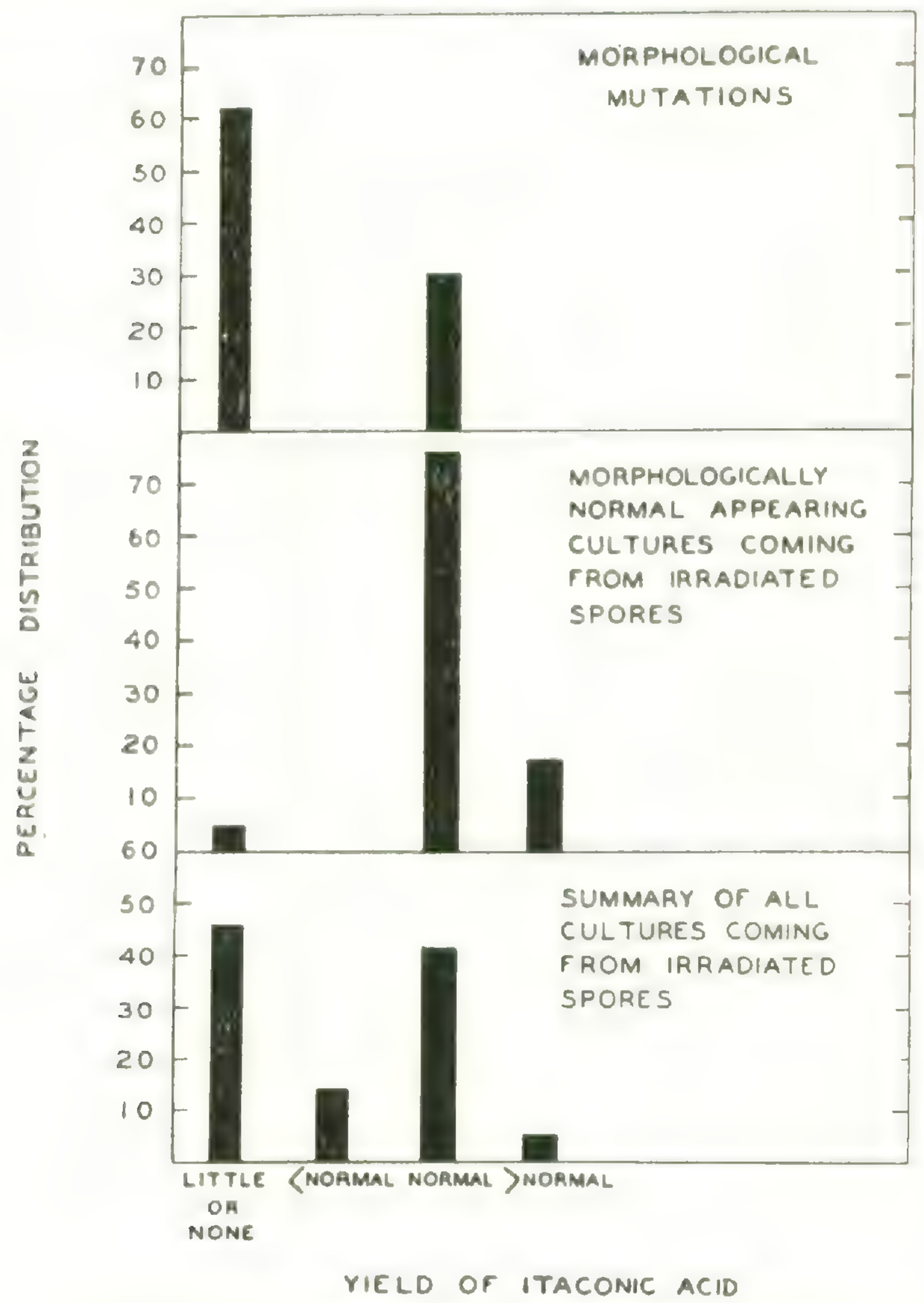

Fig. 4. Distribution of yield of itaconic acid produced by *Aspergillus terreus* mutations based on Lockwood, Raper, Meyer and Coghill ('45).

A block diagram of the percentage distribution of the tested cultures on the basis of yield of itaconic acid is given in fig. 4. The tendency of morphological

mutation to give lower yields of itaconic acid is well demonstrated.

It is not unusual to find in nature strains of *Penicillia* or *Aspergilli*, believed to represent mutations, which have different biochemical activity from the usual standard "accepted" strains; and there is good reason to expect to find in the naturally occurring strains occasionally one with more desirable fermentation properties. This type of mutation might very well have survived by natural selection. Such strains have actually been found with *Aspergillus terreus* (Raper, Coghill and Hollaender, '45). A promising investigation would be the irradiation of these new high-yielding strains and the study of the mutations produced.

If we analyze the data from these three sets of experiments, we can conclude that it is not difficult to interfere with the normal metabolism of an organism. The combination of interferences which would result in an increased production of certain chemicals can not be expected to happen often.

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THE INFLUENCE OF NUCLEIC ACID ON DEHYDROGENASE SYSTEMS A CONTRIBUTION TO THE PROBLEM OF GENE MECHANISM

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INTRODUCTION

The cellular components comprise a vast number of different compounds, salts, proteins, carbohydrates, fats, nucleates, etc., each in a state of metabolic flux, and each interacting with others to provide the energy for specific function, the supply of structural materials, and the disposal of breakdown products. This complicated and highly diverse mechanism of the cell is regulated by a mosaic of enzyme systems which are not independent but interrelated, and which are themselves regulated and affected by extrinsic and intrinsic factors and conditions. Thus, it is possible to affect the level of particular enzymes by nutritive deprivation of the cell, by the application of chemical inhibitors or stimulators, and by cellular mutations whether spontaneous or induced. Any attempt to elucidate these phenomena *in toto* is quite obviously a formidable task. The biochemist at present can study only a few systems at a time, under conditions often considerably removed from the physiological, and, as yet, with tools and concepts alike available only for first approximations. Biological chemistry owes its beginnings to the insight of men like Miescher, Kossel, and others, who believed that problems in tissue function might be at least partially explicable in terms of the chemical properties of isolated components and systems. It is with this hope undimmed, and with due recognition of the limitations inherent in the approach, that an attempt at a study of a chemical basis of gene action may be begun (Greenstein and Chalkley, '45).

Nucleic acid combined with specific proteins appears to yield conjugated compounds often with remarkable biological properties. Among these compounds are the viruses and the components of the chromosomes. Considerable information exists suggestive of a linking of nucleic acids with gene mechanisms, but in the absence of evidence for the isolated gene any attempt to identify the latter as a nucleoprotein must be treated with some reserve. Nevertheless, there is evidence that growth and reduplication are associated with the presence of nucleic acid, and the relation of the latter type of substance with the gene may thus be strongly inferred. Since the genic material exerts a controlling influence over the multitudinous functional processes of the cell, it may be assumed that it accomplishes this not by a remote form of control but by specific kinds of chemical interaction with the cellular components involved in individual reactions, i. e., enzymes (cf. Tatum and Beadle, '45). It is for this reason that the study of the chemical interaction of nucleic acid with various kinds of cellular components should yield sig-

nificant implications (Miescher, '97, Hammarsten and Hammarsten, '28, Greenstein and Jenrette, '41).

In the form of their neutral sodium salts, the nucleic acids in aqueous solution are highly elongated, polymerized molecules (cf. Greenstein, '44). The degree of asymmetry and the extent of polymerization of the desoxyribose nucleate (chromosomal component) are markedly decreased in the presence of proteins and of salts, and the relative amount of this decrease is a function of the nature, the state, and the concentration of the protein and of the salt. The molecular configuration of the nucleates is in particular highly labile toward the proteins.[1]

The question that naturally arises is whether there is some reciprocal effect of the nucleate on the molecular configuration of the proteins with which it interacts. This question is most readily answered by employing proteins with specific and readily measured properties, namely enzymes. Changes in the physical or chemical properties of these substances, due to the interaction with the nucleate, might be expected to be reflected in observable changes in their catalytic capacities. Furthermore, if such changes occurred, they would provide a possible clue to some of the mechanisms which chromosomal components exert in the maintenance and regulation of cellular functions.

PROCEDURE AND RESULTS

Aqueous tissue extracts containing reducing systems possess the capacity of decolorizing methylene blue under anaerobic conditions. We have observed that when sodium yeast nucleate (ribose nucleate) is added to such extracts, the decolorization rate is slightly decreased; when sodium thymus nucleate (desoxyribose nucleate) is added, this rate is very considerably decreased. The extent of this decrease in rate is proportional to the amount of nucleate added. Addition of xanthine results in an increase in decolorization rate (measure of xanthine dehydrogenase activity) which, at high levels of dye concentration, appears to be very nearly the same whether nucleate is present or not. The percentage increase in rate on addition of substrate, however, is greatest in the presence of thymus nucleate. Nearly identical results are obtained with freshly-mixed solutions and with mixtures which are allowed to stand until the viscosity of the thymus nucleate is reduced nearly to that of the extract (enzymatic depolymerization (Greenstein, '44)). The fact that the activity of xanthine dehydrogenase is independent of the presence of nucleate (at high dye concentrations) indicates that there is no effect of the nucleate on the dye. The results of a typical experiment are given in Table I (Greenstein and Chalkley, '45).

When the mixtures described in Table I are treated with smaller and smaller quantities of methylene blue, keeping the total volume constant by addition of water, data indicated by the curves in fig. 1 are obtained.

The exponential character of the curves in fig. 1 led us to replot the data given

[1] This configuration is also labile toward the effects of ultra-violet radiation (Hollaender, Greenstein, and Jenrette, '41), and this phenomenon is suggestive in connection with mutations induced by this agent (cf. Carlson and Hollaender, '44).

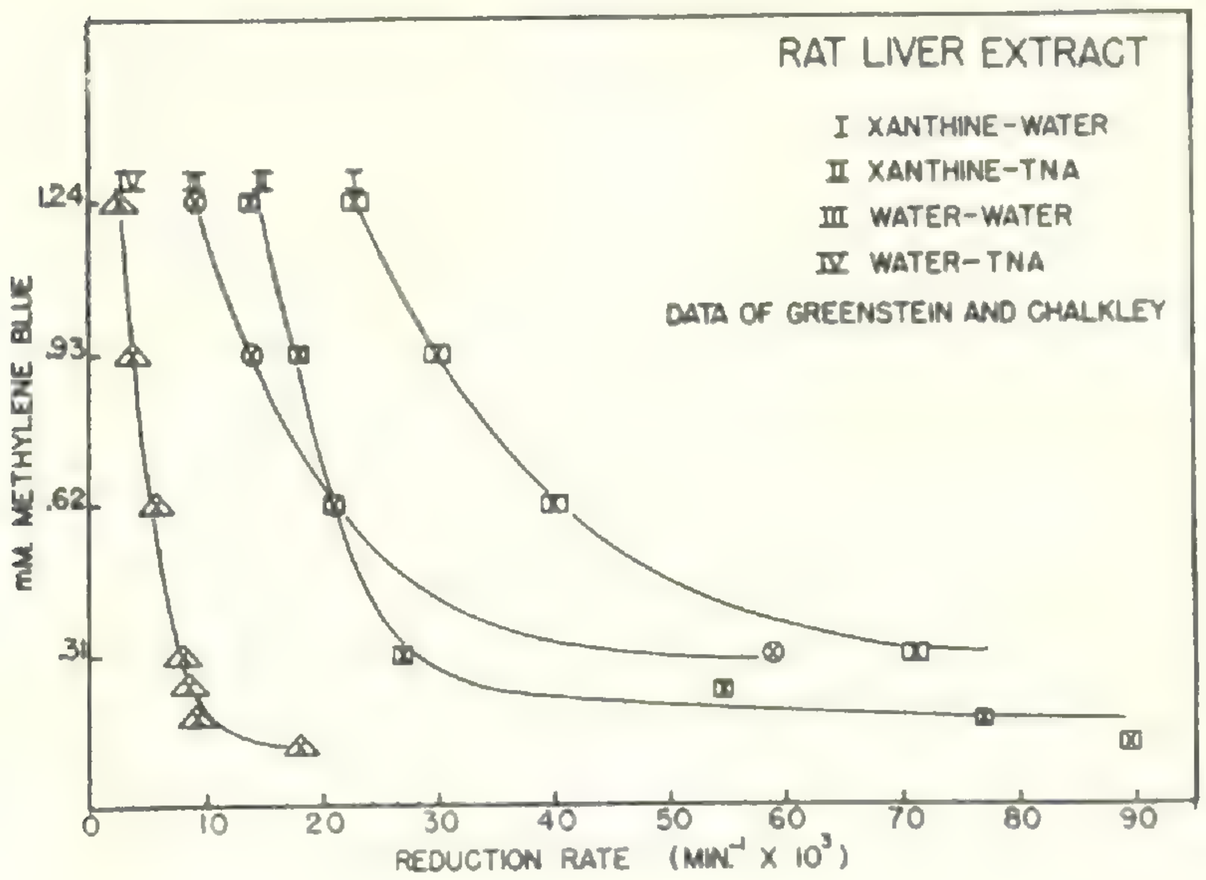

Figure 1. The relation between methylene blue concentration and the rate of decolorization of the dye. Ordinate refers to m M dye per cc. added to mixtures described by curve I consisting of 2 cc. extract + 1 cc. water + 1 cc. xanthine (1.6 m M); by curve II consisting of 2 cc. extract + 1 cc. 0.5 per cent sodium thymonucleate + 1 cc. xanthine (1.6 m M); by curve III consisting of 2 cc. extract + 2 cc. water; and by curve IV consisting of 2 cc. extract + 1 cc. water + 1 cc. 0.5 per cent sodium thymonucleate. Abscissa refers to rate of complete decolorization. Anaerobic conditions throughout. Temperature 24–26° C.

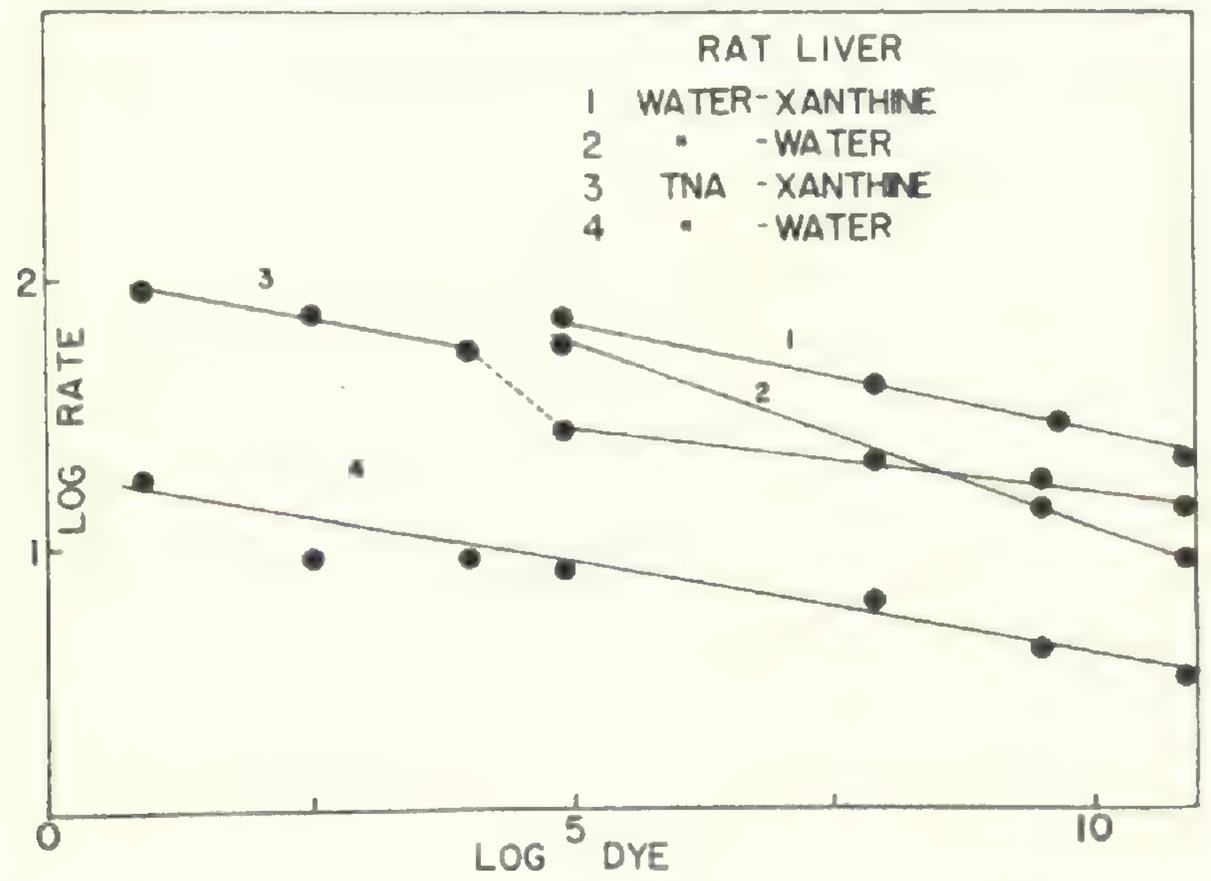

Figure 2. Relation between the logarithms of the methylene blue concentration and of the decolorization rates based upon data given in fig. 1. Composition of mixtures described by curves 1 and 4 are the same as that described by curves I and IV in fig. 1; that of curves 2 and 3 identical with that respectively of curves III and II in fig. 1.

TABLE I

EFFECT OF YEAST AND THYMUS SODIUM NUCLEATES ON THE DECOLORIZATION RATE OF METHYLENE BLUE IN RAT LIVER EXTRACTS*

Mixture†	Decolorization Rate
	Minutes $^{-1}$ x 10^3
1. 2 cc. liver extract + 1 cc. H_2O + 1 cc. H_2O + 1 cc. methylene blue	50
2. 2 cc. liver extract + 1 cc. H_2O + 1 cc. xanthine + 1 cc. methylene blue	83
3. 2 cc. liver extract + 1 cc. yeast nucleate + 1 cc. H_2O + 1 cc. methylene blue	40
4. 2 cc. liver extract + 1 cc. yeast nucleate + 1 cc. xanthine + 1 cc. methylene blue	71
5. 2 cc. liver extract + 1 cc. thymus nucleate + 1 cc. H_2O + 1 cc. methylene blue	7
6. 2 cc. liver extract + 1 cc. thymus nucleate + 1 cc. xanthine + 1 cc. methylene blue	38

* Concentration of methylene blue 1.24 x 10^{-3} M, of the nucleates 1.0%, of xanthine 1.6 x 10^{-6} M; each cc. of the aqueous liver extract equivalent to 300 mgms. of tissue; temperature 24–26° C. Anaerobic conditions throughout by employment of evacuated Thunberg tubes at approximately 18 mm. pressure.

† Xanthine dehydrogenase activity obtained by subtracting, respectively, 2. from 1., 4. from 3., and 6. from 5.

in terms of the logarithms of the respective variables with results described in fig. 2.

It is evident that there is a linear relation for all the mixtures at the higher dye concentrations, but there is a distinct break in curve 3 (fig. 2) at the lower dye concentrations. This break, symbolized by a dotted line since we do not know as yet its shape at this point, indicates an apparent increase in xanthine dehydrogenase activity. With progressively decreasing amounts of the methylene blue this increase in activity becomes progressively greater. The activity of xanthine dehydrogenase, although independent in the presence of desoxyribose nucleate at the higher levels of methylene blue concentration, is affected by the presence of the nucleate at the lower dye concentrations in such a way that it is apparently increased.

This apparent increase in dehydrogenase activity in the presence of the nucleate at low dye concentrations is all the more striking when the activity of this enzyme in the absence of added nucleate is considered. Inspection of curves 1 and 2 in fig. 1 suggests that these curves cross in the vicinity of the break in curve 3. The crossing of curves 1 and 2 means that with progressively lower dye concentrations the activity of xanthine dehydrogenase becomes apparently increasingly more negative. Further investigations have indeed revealed this to be the case. At very

low dye concentrations, the presence of the substrate, xanthine, interferes in some way with the ordinary processes of reduction of the dye by the tissue reducing systems. On the other hand, at these same low dye concentrations, when both substrate and nucleate are present, the rate of reduction of the dye is accelerated. Thus, with decreasing amounts of methylene blue, the divergence in the activity of the dehydrogenase in the presence of the nucleate or in its absence, is in opposite directions, i. e., the activity in the presence of nucleate becomes increasingly greater, the activity in the absence of nucleate becomes increasingly negative. The effect of the nucleate is thus markedly emphasized, and suggests qualitative as well as quantitative effects produced by this substance. No changes in pH are produced by the addition of the neutral sodium nucleates.

No explanation is apparent at the present time for the different directions which the activity of xanthine dehydrogenase takes in the presence and in the absence of desoxyribose nucleate. The results are quite reproducible from one extract to another. Further investigations on other enzyme systems and on other tissues are clearly desirable.[1]

Discussion

At this time any attempt at interpretation of the phenomena above described must of course be speculative. They are, from the biological viewpoint, the initial results of an attempted approach to the problem of the well-known role of the nucleus as co-ordinator of the functional activities of the cell. In this respect, it is perhaps permissible to note that, as far as the writers are aware, the results

[1] Since the above was written, more experiments involving a greater range of dye concentrations have been performed. These have shown that the relative rates of decolorization in the presence of nucleates may actually be accelerated when the dye concentration is sufficiently increased. It will be noted from fig. 2 above that curves 2 and 4 converge as the dye concentration is increased and presumably would cross at a relatively high dye concentration. The reality of such an intersection has now been experimentally established, and thus the existence of an accelerating effect of desoxyribose nucleate at sufficiently high dye concentrations has been proved. A similar study has also been made with respect to ribose nucleate, and it has been found that an analogous reversal of effect occurs, but at a substantially lower concentration of dye than that required for desoxyribose nucleate. Thus, at sufficiently low concentrations of dye the addition of either ribose nucleate or desoxyribose nucleate retards the decolorization rate; at sufficiently high concentrations of dye the addition of either nucleate accelerates the decolorization rate, whereas at intermediate concentrations of the dye the addition of ribose nucleate accelerates, and the addition of desoxyribose nucleate retards, the decolorization rate. The relative ranges of dye concentration where these three separate effects are noted will depend on the tissue used, the degree of dilution of the extract, and on the concentration of the nucleate.

Dilution of the extract may be taken as equivalent to reducing the amount of oxidizable substrate. Changes in dye concentration may be equated to normal hydrogen acceptor levels. Hence it would appear that for any given level of substrate and hydrogen acceptor the level of nucleates within the cell would determine the rate of dehydrogenation (anaerobic stage of oxidative metabolism). Since fluctuations in the release or production of nucleates within the cell may reasonably be assumed to be associated with gene action, we may be here dealing with the normal mechanism for control of general cellular metabolism—at least in so far as the initial stages of oxidative metabolism are concerned. The system or systems here studied are obviously highly complex and appear to exist in a delicately adjusted state of equilibrium. As a further example in addition to the phenomena listed above, the use of phosphate buffers normally considered innocuous, when added to tissue systems in such a way as not to disturb the pre-existing pH, markedly alters the quantitative aspects of the decolorization rates. Specific salt effects are thus also concerned in the kinetic mechanisms. Investigations are now in progress covering this and related aspects of the problem.

constitute the first direct chemical evidence that a typically nuclear constituent, desoxyribose nucleic (thymonucleic) acid can directly affect the enzymatic redox systems of the cell.

The fact that, in the presence of desoxyribose nucleate, the over-all activity of these systems is altered immediately suggests that differences in production or release of such nucleates within the cell could regulate its metabolic activities. It also appears, since the effect of the nucleate is apparently relatively, if not absolutely, reversed for xanthine dehydrogenase (the enzyme typically involved in purine metabolism) at low oxidative levels (i. e., low concentrations of methylene blue), that this regulative effect might extend to the metabolism of the nucleic acid itself. In that case the effect would apparently depend largely upon the concentration of the hydrogen acceptors available, up to and including oxygen.

One is tempted to recall the finding of Chalkley and Voegtlin ('40), that changes in oxygen tension strongly affect the growth and fission of the nucleus in *Amoeba proteus* and also the sulfhydryl cycle observed by Chalkley ('37) within the nucleus of the same organism, which last could conceivably produce a variation of redox equilibria within the nucleus, i. e., in the immediate vicinity of the desoxyribose nucleic acid, and which was shown to be correlated with the growth and fission of the nucleus. Considered in conjunction with the present data, these observations might lead to the suggestion that the oxygen supply together with desoxyribose nucleic acid metabolism might, through affecting the redox systems of the cell, constitute the regulating mechanism of the entire cell metabolism.

Further, the intimate relation of the desoxyribose nucleic acid to chromosome structure might suggest that gene activity expresses itself in no small part by means of this system.

As stated above, this is obviously speculation but at least there can be no doubt that we have at last definite physiologic, i. e., biochemical, action demonstrated to occur between a component limited to the nucleus and certain enzyme systems concerned in cell metabolism.

It is of interest to note that the ribose form of the acid (yeast nucleic acid) shows, if any, much less retardative activity. Thus the cytoplasmic form of the acid is sharply set off in this respect from the purely nuclear form.

The implications of these extremely simple experiments are far-reaching. It is obviously necessary to carry out greatly extended research, however, before any of the foregoing suggestions can have any merit other than that of serving as indications of our mode of approach.

SUMMARY

Aqueous extracts of rat and of mouse liver possess the capacity of reducing solutions of methylene blue. In the presence of desoxyribose nucleate the rate of decolorization of relatively low concentrations of dye is appreciably delayed, and the decrease in rate is proportional to the nucleate concentration.

At relatively high levels of dye concentration the activity of xanthine dehydrogenase in these extracts is the same whether desoxyribose nucleate is present or not. At low levels of the dye there is an apparent increase in the activity of the enzyme when desoxyribose nucleate is present.

Ribose or desoxyribose nucleate may accelerate or retard the reducing systems of the liver, the effect depending on the dye concentration.

The possible implications of these findings are discussed in the light of genic mechanisms.

Acknowledgment is made of the skillful and devoted assistance of Miss Florence Leuthardt.

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GENETIC ASPECTS OF VIRULENCE IN BACTERIA AND VIRUSES[1]

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At Iowa State College we consider that disease is due to the interaction of four major variables: the genetic constitution of the host for disease susceptibility or resistance, the genetic constitution of the pathogen for virulence or avirulence, the dose of the pathogen to which the host is exposed, and a multitude of variables which we ordinarily include in any genetic experiment under the head of environmental effects. For disease to be produced, the genetic constitution of the host must be a mirror image of that of the pathogen in that a genetic constitution for susceptibility has a relatively low survival value against all organisms whether they are virulent or avirulent. A host constitution for medium susceptibility has a fairly high resistance against avirulent organisms, medium resistance against medium virulent organisms, and a small resistance to the highly virulent type. A highly resistant host has high resistance for all organisms except the most virulent to which they now and then succumb.

HOST MATERIAL

The studies herein reviewed were started in 1925 by differentiating a single host strain for mice and for the domestic fowl into forms highly resistant to *Salmonella typhimurium* and *Shigella gallinarum* respectively. From earlier experiments a dose of 5×10^4 organisms per mouse was chosen as the agent by which resistant strains were established from the previously highly susceptible strains. Similarly for poultry a dose of 1.2×10^7 of the fowl typhoid organism *Shigella gallinarum* was chosen. These organisms were inoculated intraperitoneally. Animals which survived in the best condition in each generation were used as the parents for the next generation. The results of the first fourteen successive generations of selection are shown in fig. 1. Intense inbreeding was used for each group to purify the genetic constitution.

The graphs of fig. 1 show that for both hosts the resistance increased rapidly at first, then somewhat more slowly for six or seven generations, the ultimate survival value of each group being 80 to 90 per cent. In the eighth generation for the mice the dosage of organisms was increased to 2×10^5. This increase was accompanied by a 10 per cent reduction in survival. From that point on the resistance increased again, 93–96 per cent resistant animals being reached in the 14th generation. The results show that despite continuous selection and inbreeding for eight generations there was further residual variation within the strain. Chicks of the eleventh generation were not tested. Subsequent tests showed high

[1] Received for publication, February 24, 1945. Journal Paper No. J-1270 of the Iowa Agricultural Experiment Station, Ames, Iowa. Projects No. 251, 252, and 573.

This work was aided by a grant from the Rockefeller Foundation.

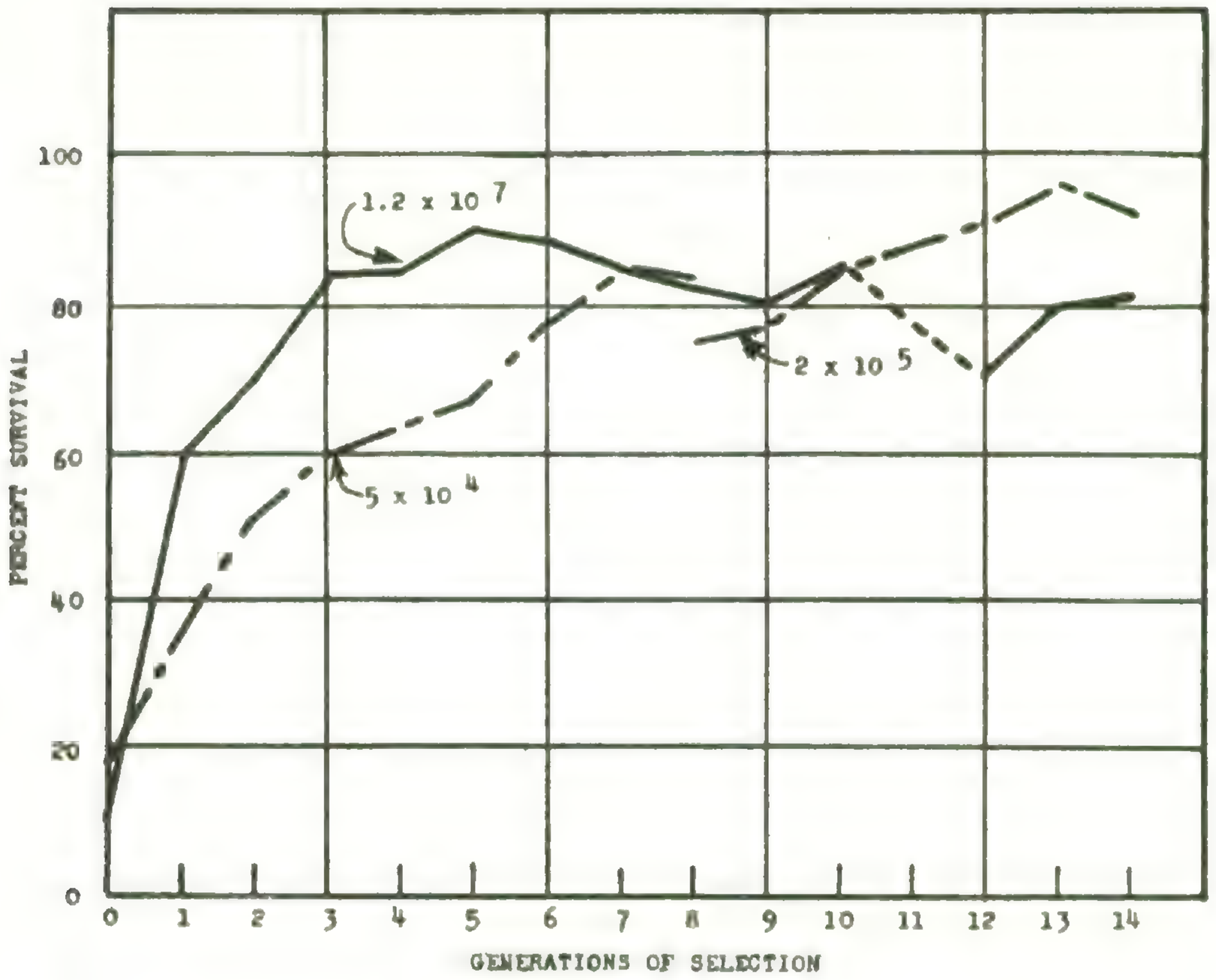

Fig. 1. Survival value of mice (Schott, '31, '32 and Hetzer, '35, '37) and the domestic fowl (Lambert, '31, '36) for successive generations of selection toward the resistant types. Solid line for fowl, dot-and-dash line for mice. Eleventh generation of fowl selection was untested. Eighth generation of mice had dosage changed from 5×10^4 to 2×10^5.

resistance within the chickens. Testing of the parents is not necessary for the resistance in the progeny. The chickens and mice have kept their high genetic resistance despite the fact that the inbreeding has led to the accumulation of genes for smaller size, lower fertility, and some apparent lack of vigor.

Two possible explanations might account for this change in resistance: selecting of small variations due to genes for resistance already in the parental lines or selecting of mutations toward higher resistance, each mutation being partly dependent on the total genetic constitution of the host for disease resistance. Either explanation would lead to the disease resistance observed in these lines. Actually both factors appear important. There is some experimental evidence to indicate that if a sufficiently large population of mice is chosen it is possible to pick out from this population individuals which carry very high genetic resistance to mouse typhoid and actually make the change from a relatively susceptible population to a highly resistant population in a very few generations. This sudden change could favor the view that disease resistance may be accomplished in one step or may be due to a single gene pair. The results of Hetzer show that this is not the case. The circumstances leading to the choice of resistant mice are fortuitous, a result of a combination of several genes for resistance in one animal brought about by chance segregation.

A second question of interest is why a completely resistant strain is not attained. The strains which have been formed would be considered completely resistant if bacteria of low virulence were used to initiate the disease. With highly virulent organisms, some deaths do occur. It seems that no species of animals having a native disease has yet had a completely immune race established through genetic means or any other means for that matter. Highly resistant animals have been produced but with highly virulent organisms of the pathogen it is possible to produce some deaths in all cases.

Since the fourteenth generation the selected strains have been maintained without testing. The resistance of the present generation is as high as it was under testing. Genetic resistance when made homozygous for the strain is a permanent attribute of the strain.

PATHOGEN VARIATION

In November, 1940, our culture of *Shigella gallinarum* was found to have completely lost its virulence when used in a fairly large test. This culture had been highly virulent the previous May. The results demonstrate that a large population of virulent bacteria could be replaced by avirulent bacteria in a period of something like seven months. This fact was of particular interest since this line had previously retained its pathogenicity for several years.

The mechanism of such changes was not entirely unknown to us as our previous studies of such genetic variation of the pathogen had given us a fair understanding of how they came about. The following investigators made large contributions to this problem and to the others herein discussed. The results reported are the joint effort of the following workers in our laboratory: Dr. M. R. Zelle, Miss Janice Stadler, Mr. G. W. Kohler, Mr. John A. Weir, Mr. A. E. Bell, Mr. E. F. Oakberg, and Dr. R. E. Lincoln.

The avirulent culture was subjected to the proper cultural and serological studies to prove that it was *Shigella gallinarum*. The culture was then inoculated into a chicken of a very susceptible strain. Five different isolations were made from this host. Two of these isolations did not progress very far, one being lost in the first passage and the other one shortly thereafter, indications of the low virulence of the *Shigella* culture.

Isolations were made from the heart, liver and spleen respectively. As no small chicks were available, these lines of bacteria were passed through six successive 10-week-old birds of the susceptible strain, each line being kept separate from the others.

The bacteria were kept in the chickens one week, then for three days on culture media at each passage. The inoculating dose was two billion organisms. These passage birds showed no mortality, but the organism was recovered from each bird inoculated. Tests for virulence on the seventh passage organisms were made on 10-day-old chicks. One line, D7, killed 10 out of 10 chicks in less than 10 days. The second line killed 6 of 11 chicks but took 21 days to do it. The third line

killed 8 of 11 chicks but also took 21 days to do it. A transfer culture of the parent avirulent culture from which the above lines originated killed 3 of 11 chicks in 21 days. It is evident that one of these strains, D7, differs from the others in virulence.

Further analysis of line C showed that for an average dose of 5×10^6 it killed only 6 out of 35 resistant chicks and 11 out of 37 susceptible chicks. Line D killed 13 out of 41 resistant chicks and 36 out of 36 susceptible chicks. Line E killed 11 out of 40 resistant chicks and 34 out of 34 susceptible chicks. The percentage comparisons were for the resistant line 17, 32, 27, and for the susceptible chicks 30, 100 and 100, for lines C, D and E respectively. The parent avirulent culture showed 24 per cent mortality in the susceptible host. Two relatively pathogenic lines had been established from a highly avirulent line. The mechanism of this selection is important.

To determine the variability of *Shigella gallinarum* under natural conditions, a survey was made of cultures from chickens diagnosed as clinical fowl typhoid during the summer. Sixteen cultures and 11 sub lines showed marked variability in the end point for agglutination in anti *Shigella gallinarum* and/or anti *Salmonella typhimurium* serum, metabolism of sugars, colony morphology and pathogenicity. The species *Shigella gallinarum* evidently had wide genetic variability.

ANALYSIS OF VIRULENCE CHANGES

Experiments were planned to analyze bacterial variability as it is related to the genetics of virulence. From the avirulent stock culture described above 20 colony isolations were made. As this organism does not clump appreciably, each of these colony isolations probably represents the descendants of a single bacterium. Ten of these avirulent lines were exposed to the environment of our inbred, highly resistant chickens described above. These inbred lines are capable of surviving nearly 1000 times the number of bacteria which will cause death in most flocks. The other ten strains of avirulent bacteria were grown in a strain of chickens marked by susceptibility to fowl typhoid. Two chicks were used at each passage for the resistant host line and one chick for the susceptible host line. The avirulent strains of *Shigella gallinarum* were thus exposed, on the one hand, to the intensely unfavorable environment of the resistant strain of host, and, on the other hand, to the more favorable environment of the highly susceptible host.

Attempts were made to pass each culture successively through 16 different 10-day-old chicks using the technique described above. Despite the fact that twice as many chicks were available for recovering the organism at each resistance passage, 24 passages were lost in the transfers through the resistant host compared to 10 for the susceptible series. Life for the typhoid bacteria in the resistant host was tough. The avirulent strain has great difficulty in establishing itself even to making a mild disease in the resistant host strain. This fact suggests that the resistant host would be a potent selecting force tending to pick out the progeny

of any variants characterized by increased virulence.

Small tests were made throughout the passage experiments to determine the constancy with which the organisms recovered retained their virulence. A larger test was made at the end of the experiment to establish more exactly the virulence of each line. The rather scattered and low amount of data taken during the passage of the 20 lines of bacteria through their respective hosts show that each strain retained its low virulence for a varying number of passages. Changes when they did occur came suddenly during a single passage and resulted in a substantial gain in virulence, the total amount of change differing for different strains. When a change in virulence did occur, the subsequent tests showed a retention of the new virulence. These results favor mutation and subsequent replacing of the avirulent type by the virulent mutant.

Tests of the 20 lines at the end of the sixteenth passage give further support of this conclusion. Two of the lines, I and R, had not changed in virulence as the result of growing in their natural host for half a year (fig. 2). One line was carried in the resistant host. The other line was passed through the susceptible host. If virulence is due to chance mutation, the expectation would be essentially equal numbers of mutations in each group. The observations bear out this hypothesis. Two lines of medium virulence have been established from the resistant host against three lines for the susceptible host. Seven highly virulent lines came from resistant host passage and six from susceptible host passage.

The over-all picture for the 10 lines passed through the resistant hosts was as follows: A dose of 100,000 organisms inoculated into 74 resistant chickens led to 20 per cent death; inoculated into 203 susceptible chickens led to 70 per cent death. With 100,000,000 organisms as the dose, 70 resistant chickens had 22 per cent death, 124 susceptible chickens had 88 per cent death. For the lines derived by passage through the susceptible host the 29 resistant chickens with 100,000 dosage had 7 per cent death and the 186 susceptible chickens had 84 per cent death. For the 100,000,000 dosage 54 resistant chickens had 22 per cent death, and 98 susceptible chickens had 86 per cent death. These data show that passage through either host is equally favorable to establishing of virulence. The degree of increase in virulence may be judged by the fact that the original avirulent culture inoculated in 100,000,000 organisms showed no death on the resistant host and only 34 per cent on the susceptible host.

The chicks of either strain are highly efficient selective agents favoring any variants toward virulence and encouraging them to multiply at the expense of the avirulent type. The population within the host becomes rapidly purified towards the virulent type. The genetic constitution of the domestic fowl, the natural host to this disease, is sufficient to create the necessary conditions for this selection process. The culture media, on the other hand, appears to favor those organisms whose genetic constitution is for a saprophytic type of growth.

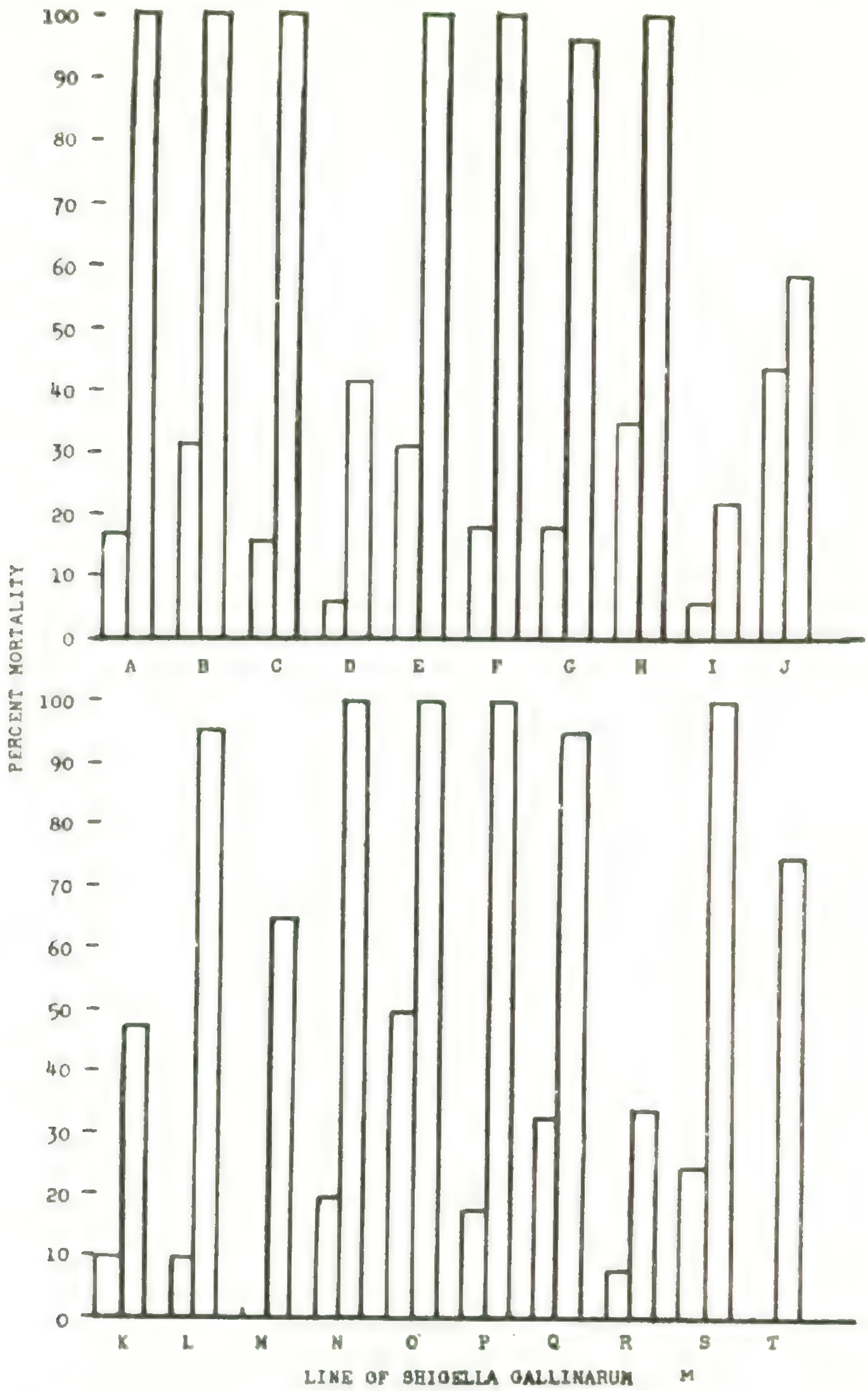

Fig. 2. Virulence of different lines of *Shigella gallinarum*, originating from the same avirulent line, after 16 passages through resistant chicks A to J and susceptible chicks K to T. Left, ordinate tests on resistant chicks; right, ordinate tests on susceptible chicks.

GENETICS OF VIRULENCE IN MOUSE TYPHOID

Experiments of a similar nature were carried on earlier in this laboratory utilizing *Salmonella typhimurium*, the agent of mouse typhoid. A single laboratory line of *Salmonella typhimurium* was available. This line had retained constant virulence on culture media for more than ten years. Six different experiments were performed, each varying somewhat, but all directed toward detecting and tracing virulence changes in this line. Six different strains of mice, differing in their resistance to mouse typhoid, were available.

In some experiments the bacteria selected for use were the direct descendants of a single organism picked out by the micropipette. In others, the organisms were the result of five successive platings and single colony isolations. The initial bacterial line chosen had essentially the same virulence as the parent culture. The parent culture was different from that described for the domestic fowl in that it was originally a culture of medium virulence.

The culture was divided into two parts: one part exposed to the effects of the environment of the resistant host strains of mice, the other part to the environment of susceptible host strains of mice. The longest experiment performed involved 36 successive passages of bacterial line from one mouse to another and covered a period of two years. The outcome of these experiments brought out several facts important to our interpretation of the physical basis for virulence of a disease-producing organism.

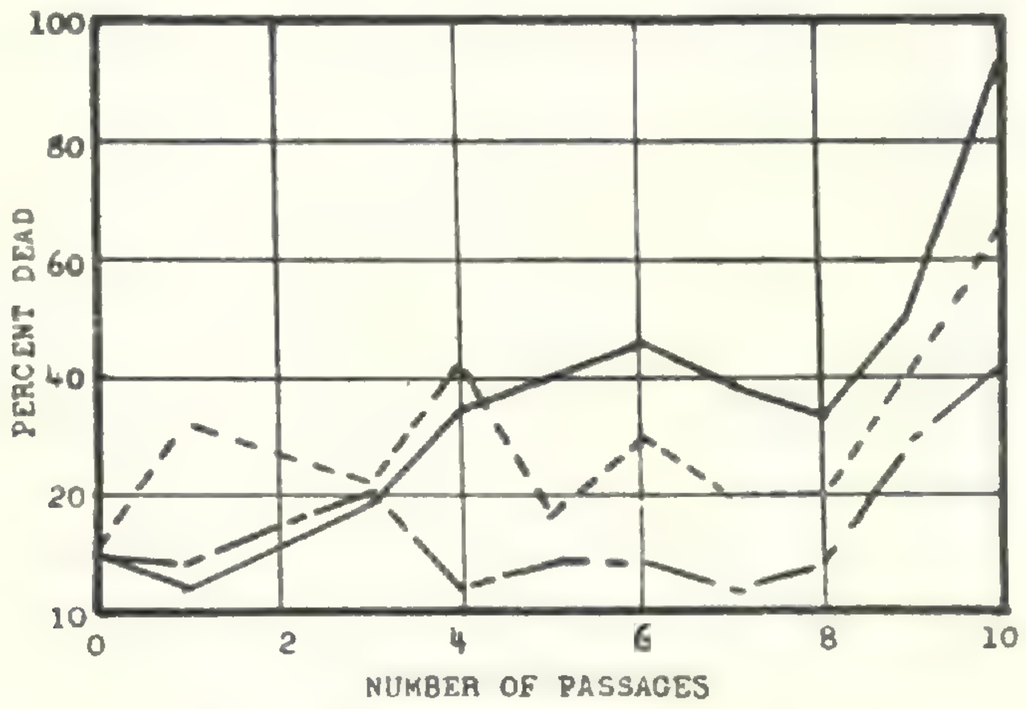

Fig. 3. Changes in virulence observed in the passage of a medium-virulent line through susceptible mice, through resistant mice, and as kept on culture media. Solid line is for the passage through resistant mice; dotted line, for passage through susceptible mice; and dash-dot line, for the culture on culture media. Tests made on resistant mice.

In general, the stability of our original pathogenic line was demonstrated. Increased virulence, when it was observed, occurred only in a low proportion of cases following passage of recently isolated single-celled cultures. Changes in virulence, when they did occur, were sudden. The increased virulence observed was then subsequently maintained at the new high level. There was no suggestion of a gradual accumulative effect of the environment on these increases in virulence. Lines of the pathogen more virulent than the parent culture were obtained in passages through both resistant and susceptible hosts. If virulence increased during passage, the increase was abrupt. Continued growth in the particular host environment resulted in no further increase in virulence. Figure 3 shows one of these experiments.

As bacterial lines were isolated they were sometimes marked by morphological characters along with virulence differences. This association suggests that the phenotypic character of the bacterial colony may be an expression of its virulence.

The correlation is high but not complete. Most but not all of the lines isolated and showing increased virulence went toward the smoother types.

By making use of two bacterial lines differentiated in their colony phenotypes, as well as by their virulence, it was possible to show a very intense selection for the more virulent line of bacteria. Bacteria from the two lines were mixed in definite proportions and then inoculated into mice. At intervals after inoculation bacteria were recovered from the mice, and the proportion of the two types determined. The results showed that the virulent forms quickly became dominant and often were the only type present in the population.

A further confirmation of this fact comes in experiments in which virulent bacteria are inoculated as against those in which avirulent bacteria are used. The bacteria are recovered easily from the host inoculated with virulent culture but with difficulty when inoculated with avirulent organisms.

By growing a large progeny of a single-celled isolation on agar media, it was possible to isolate five different phenotypes appearing as variants of the original culture. Four of these variants showed colonies of greater roughness than the parent. In one the colony was smoother than the parent. This line was also unstable in its phenotype, occasionally producing rough variants. Tests of these phenotypic variants showed that some differed from the parent culture in virulence. The observed facts thus demonstrate the occurrence of phenotypic variations in the progeny of single cells. The amount of this variation is sufficient for the changes in virulence observed in our experiments. Changes in virulence in artificial media are of the same type as those observed in the host. They appear suddenly with sharp differences between the phenotypes.

No relation was observed between the virulence, growth rates, or fermentation reactions of the lines.

In essence the results show changes in virulence to be analogous to mutations in higher forms. The rate of mutation for a given type is small. The changes are sporadic in their appearance and when they do occur are permanent and true-breeding. The variation can go in each direction—toward higher virulence or toward avirulence. The environment of the host or culture medium acts as a selective agent for the genetic type which fits the environment. The environment is not the cause of the variation.

MUTATIONS IN *PHYTOMONAS STEWARTII* AND THEIR RELATION TO VIRULENCE

The problem of virulence and its dependence upon the inherited bacterial constitution may be studied by searching for phenotypical variants in an original pure stock. These variants may occur naturally, under irradiation or in other ways. Two different lines of a corn-wilt organism *Phytomonas stewartii* have been examined for mutations which occurred naturally and after irradiation with X-rays. The first of these lines is a dark yellow rough type with a medium-sized colony. The second type is a large colony with diffuse center. The virulence of these parent lines may be judged by comparing the green weight of plants inocu-

lated with them as contrasted with that of the normal uninoculated plant. Twelve mutants of the dark yellow rough type parent were compared in virulence with the parent type. These mutants were of several kinds. The colonies might be pale yellow, white, roughs of several grades, extreme smooths, mucoid or dry, large or very small. Some of these mutant types, photographed at the same scale and age, are shown in fig. 4.

One parent type had a virulence index of 31, or it was rather low in virulence. Of its 12 progeny mutants 3 were below the parent and 9 were above the parent in virulence. The virulence indexes ranged up to 70. The average was 45. Virulence variations sometimes accompanied the morphological variations and were apparently an expression of the sudden change in type.

Eight variations from the other parent type were selected on the basis of like characters. This parent type had a virulence index of 75. Seven of the 9 mutants tested showed virulence indexes below that of the parent, ranging to as low as 46. Two had indexes above the parent, 81 and 78. The average virulence was 62. The abrupt phenotypic changes in bacterial type observed in this line likewise may affect virulence.

Certain apparent correlations are evident in this comparison. The mutants tend to remain fairly close to the parental type in their virulence. The variation which is observed seems to be directional. When the original parent stock is of rather low virulence, a mutant is most frequently of a somewhat more virulent type. When the parent is of virulent type, then the mutants tend to show less virulence than the parent.

VIRUS MUTATIONS AND PATHOGENICITY

Several investigators working with viruses have noted changes in strain type and in pathogenicity. The analysis of these changes has come particularly in the study of the tobacco mosaic viruses where McKinney noted that suddenly appearing yellow types might be due to mutation from the original form. Jensen isolated over fifty of these variant types occurring normally in ordinary tobacco mosaic. During the course of our own studies on tobacco mosaic large numbers of different variant types have been obtained. These types may be grouped into three major categories: those similar to ordinary tobacco mosaic, those similar to aucuba mosaic, and those producing yellow-mottling rather than the green type. Besides these differences, there are quantitative variations in invasive capacity which seem to be characteristic of the individual variants.

Attempts have been made to determine the inactivation rates of some of these mutants under similar X-ray treatments. The results of these studies indicate that within the limits of accuracy of the X-ray determinations the inactivation rates of the different mutations are the same as those for the parent type. As the different variant types originated from the same parent type, it follows that in so far as this property is concerned, it has been preserved in the variants while they have varied in other directions, i. e., invasive power or phenotypic expression of the host plant. If we take the view that inactivation rates under the same

conditions measure the reproductive size of the virus, it would follow that the size of the virus particle had not changed while the mutation was taking place. The mutation could not be accounted for as splitting of the original particle or as a polymerization to a larger size. Thus the virus may mutate in one characteristic while its other characteristics remain the same as the parent.

Holmes has made a most important study along these lines. He has studied the mutations derived from a masked strain and from a distorting strain of tobacco virus. Thirty-one yellow variants from the distorting strain and 84 variants from the masked strain were observed. Twenty-three out of the 31 variants from the distorting retained fully the systematic invasive power of the parent type. But three of the variant strains produced only local lesions. The mutants from the masked strain, on the other hand, produced no fully invasive types in the 84 which were examined. The changes to the yellow mosaic type in the variants are independent of the invasive characteristic and may represent unit differences in the structure of the viruses similar to such differences in particular genes. These results agree with ours on *Phytomonas* in showing a persistence of virulence type even with marked changes in other characteristics.

These facts gather added significance if the hypothesis that each of these viruses is a unit or molecule is accepted. It would mean that an individual virus may have several side chains or like structures which are capable of affecting the host phenotype in different ways. It will be remembered that tobacco mosaic particles take the form of a long rod composed of smaller units seemingly repeated throughout its length. A single particle could then get its different properties either by different structures of the whole or by having each unit so differentiated as to be responsible for a given reaction.

Each of these different variants of a parent type evidently retains the common characteristics of the capacity for self-reproduction. Added to this basic character, permanent structural alterations may lead to yellow vs. green mottling or localized necrotic lesions vs. the spreading invasive type, etc.

The reproductive capacity is subject to modification by radiant energy and other means. Loss in infectivity may take place under the action of X-rays or ultraviolet light without changes in other properties sufficient to be detected by serological or other means. The presence of these properties is not an indication of the capacity of the virus to reproduce.

Serological techniques of precipitation, complement fixation, or virus neutralization may be used to distinguish different viruses from each other as shown by Chester and others. Strains within a given virus are not readily detected by these means even though these strains show markedly different phenotypic characters in the host plant. These facts indicate that various changes may occur and not be reflected by serological reaction. This may be expected if the particular alteration necessary to produce the new type is not of an antigenic nature. On the other hand, the fact that the larger differences of tobacco mosaic and aucuba mosaic and the enation mosaic may sometimes be detected by serological means indicates that at least some of the changes within these forms are antigenic in nature. One

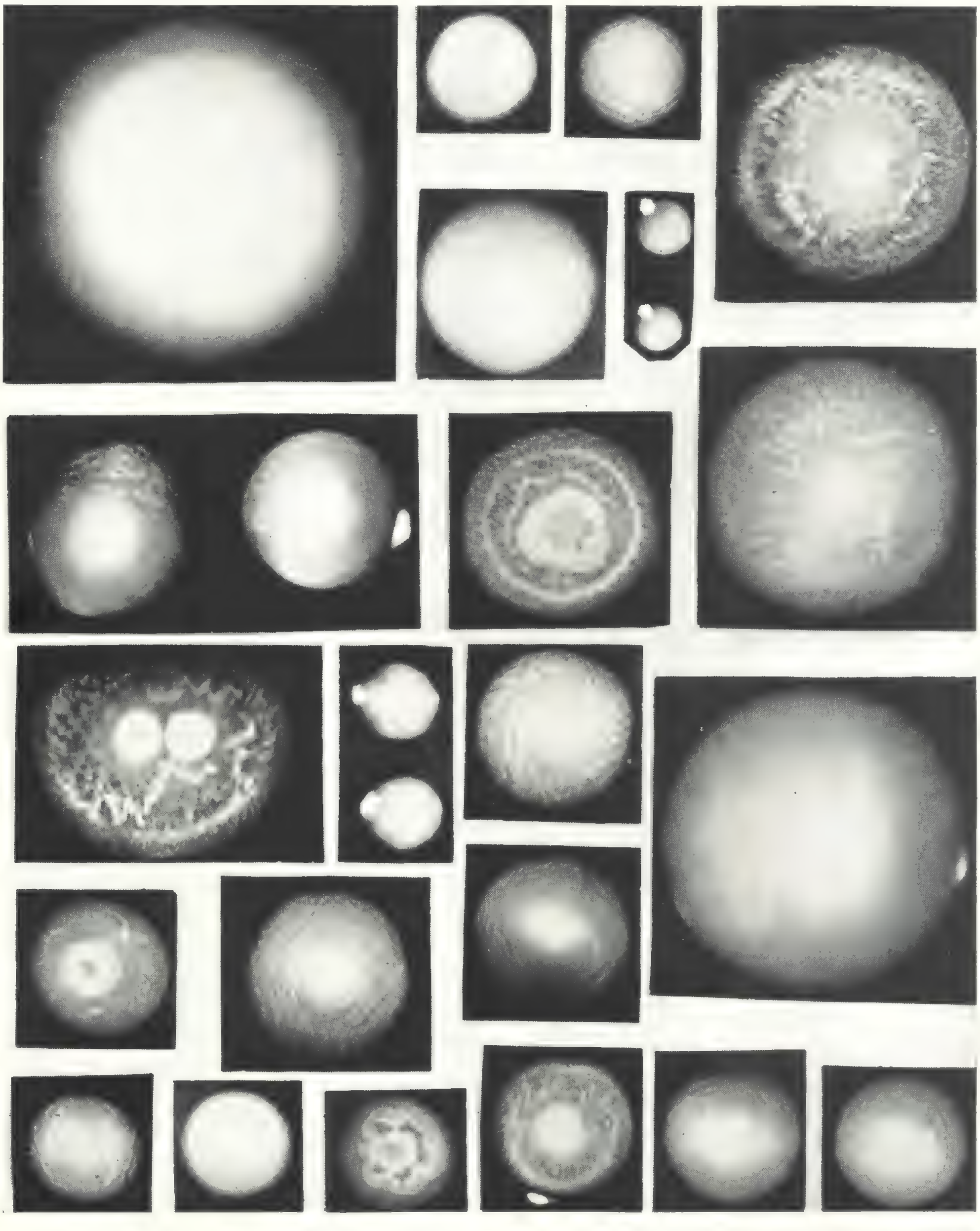

Fig. 4. Mutant types from two lines of *Phytomonas stewartii* differing in virulence.

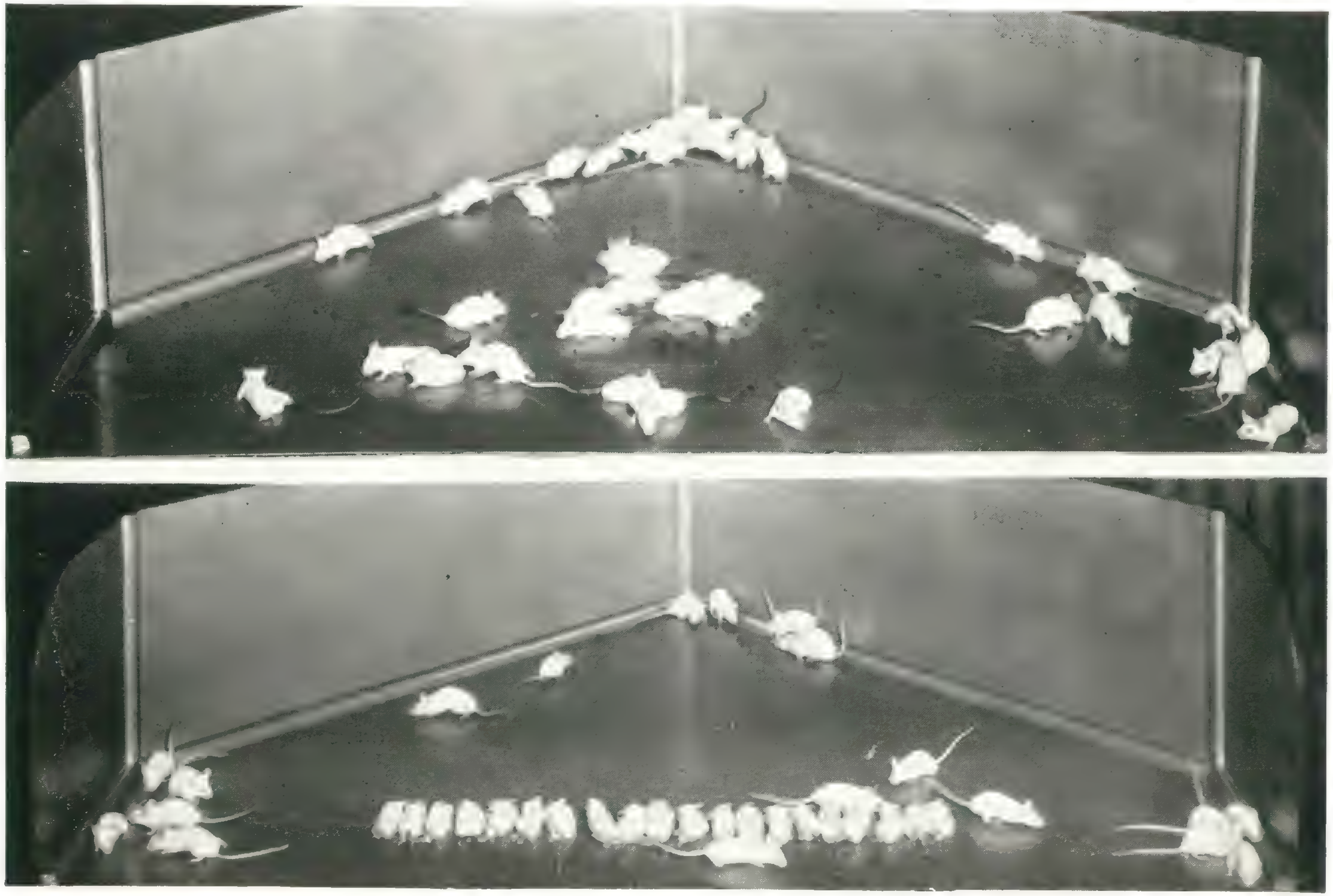

Fig. 5. Photograph showing 20 mice each of the S and Ba strains inoculated with like dose of the same typhoid culture. All the S mice survived; all the Ba mice died.

might assume that such changes involve some type of polysaccharid rearrangement. These facts have a direct bearing on the gene and the detection of its mutants. They suggest that extreme variants of the gene might be detected by a change in its serological structure. Less extreme variants appear much less likely of detection. Under such circumstances the products of the gene's action rather than of the gene's own structural alterations appear more likely of detection.

ORIGIN OF VARIANTS

Variants which occur in cultures *Shigella gallinarum, Salmonella typhimurium* and *Phytomonas stewartii* appear to fall in two distinct categories: those in which the variant is stable from the time of its appearance and those in which the variant breaks up into two types, one of which is stable, the other of which continues to break up into the two types in successive generations. These two categories appear to form a discontinuous series and there is reason to believe that the mechanism behind the changes may be distinct.

The time of bacterial mutation in *Salmonella typhimurium* has been traced under microscopic observation. A smooth mutant type was chosen. This type is called unstable as its colonies are composed of bacteria which, on one hand, give smooth colonies and, on the other, give rough colonies. Bacteria from the rough colonies give nothing but rough. The smooth type will repeat, giving both smooth and rough colony types. The case is therefore comparable to similar unstable mutant types in *Drosophila*.

A single organism of the smooth type was picked up in a micropipette, and placed on a thin agar film under the 4-mm. power of the microscope. This cell divided and the daughter cells were separated from each other with the micro-pipettes. The cells divided again and were again separated. In this manner it was possible to separate and mark the individual cells of six successive divisions. The cells were then allowed to grow into micro-colonies. Each micro-colony was separately picked off, the cells separated by shaking in liquid media and then seeded on agar plates to identify their types. If the colonies were of three types—smooth, mixture of smooth and rough, and rough—the original cell was the smooth unstable type. If the colonies were all rough, the original cell was a mutant to the rough type.

In two such pedigrees a single mutant rough colony was observed, a rate of change of 1 in 134 cells. The sister cell to the rough mutant was smooth. The fact traces the mutations to the events occurring in the division of a single cell into two cells. It shows that the change is not due to any over-all environmental effect for even sister cells do not share the same effects.

The rate of mutation may be checked by a statistical analysis of the relation between the smooth type and rough type found in a single-celled culture after a certain lapse of time where the generation time is known. This statistical analysis will not, of course, substitute for the visual analysis above in showing that the variants occur as one daughter cell of a pair at a single division. Ten separate single-cell isolations were analyzed for their rates of mutation.

The results of this comparison give an average estimate of the mutation rate of 0.0053, or 1 mutation in 187 cells. This agrees rather well with the estimate from the pedigree cultures of 1 in 134 cells.

Observation of a variant appearing as the result of a single cell division has thus been made. The rate of occurrence of these variants is, however, much higher than that observed in the stable variants. An examination of temperature effects on the rate of occurrence of these variants indicates that the mechanism involved for the unstable type as contrasted with the stable may be quite different. In fact, it may be similar to that observed in crossing over or in variegation in *Drosophila*. The results make it highly probable that there are at least two methods by which the variants in bacteria occur.

HOST CONSTITUTION AS RELATED TO BACTERIAL CONSTITUTION IN DISEASE

Three lines of mouse typhoid have been preserved as the result of the above experiments. One is highly virulent; the second is of medium virulence; and the third is nearly avirulent in our customary dosage of 200,000 organisms. The host in which these bacteria have worked have likewise been segregated into strains, the survival value of each particular strain being shown in Table I. Photographs of the outcome of a recent experiment involving 20 mice each of the S and Ba strains strikingly illustrate these differences.

TABLE I

COMPARATIVE RESISTANCE OF Ba, L, E, Z, RI, AND S STRAINS OF MICE TO *SALMONELLA TYPHIMURIUM*, STRAIN 11c, INOCULATED WITH 200,000 ORGANISMS, DATA 1938 TO 1942

Strain	Lived	Total	Survival %
Ba	35	452	8
L	63	470	13
E	292	555	53
Z	733	1262	58
RI	372	496	75
S	988	1150	86

The resistance differences in these strains are genetic. They have been segregated into them and made relatively pure by various means: inbreeding, selection and inbreeding, etc. The strains have held their respective resistance levels for eight or more generations when tested with the same line of mouse typhoid bacteria.

THE CHARACTER BASIS OF VIRULENCE

From the mutations in virulence described above, three lines of the pathogen were preserved. One line is highly virulent, a second has medium virulence, and the third is nearly avirulent in our customary dosage of 200,000 organisms. Hypothetically, virulence in a pathogen could be due to (a) an accentuated capacity for growth to a point where sheer numbers overwhelm the host, or (b) the organism having the capacity to produce a toxin to the host. Some insight into this question was obtained by comparing the lethal action of living and heat-killed cultures (56° C.) of our three bacterial lines. Under these conditions the six strains of mice retained a similar order of resistance to the heat-killed organisms that they had to the live organisms. This indicates that resistance to toxic substances produced by the pathogen is certainly one of the characters involved in genetic resistance or susceptibility. Post mortem examinations, showing that the dead organisms may produce sterile lesions comparable to those observed in the same strains inoculated with the same line of living pathogens, give further support to this view.

However, capacity to grow in the host is also a factor. This is shown by the comparison of the mortalities for the three bacterial lines, when alive or dead. Two of the bacterial lines, differing markedly in live-organism virulence to all mouse strains, showed little difference when compared on a heat-killed basis. Growth rates of the three bacterial lines are equal so that rate of growth of the organism is presumably not a factor. Rather it is the capacity of the organism to grow in the host to numbers which will be lethal. The most lethal bacterial line can grow in mice to the toxic limit. The second pathogen is stopped by the host's resistance before this point is reached. Two genetic characters are thus of demonstrated significance to pathogenic bacteria; the capacity to elaborate toxic products and to multiply rapidly in the host.

CHARACTER BASIS FOR ACTIVE AND PASSIVE IMMUNITY

Mice from the six strains were immunized by inducing clinical typhoid by the live-organism route. The three bacterial lines were used. These mice were subsequently inoculated with a large dose of a single bacterial line. The follow-up dose of live organisms necessary to get a fair death rate is about the same as that necessary to get similar death rates in immunized mice treated with killed organisms. This fact indicates that although antibodies present in the immunized host are able to check the growth of moderate numbers of pathogens, the ability to withstand the toxins produced by the bacterial cells is not greatly altered by the previous immunizations.

But individual host-strain differences appeared on immunization. Using a factorial design some 2700 mice were immunized with killed cultures of three different lines of our bacteria. The mice were equally divided among three different strains, one highly susceptible to typhoid, another of intermediate susceptibility, and a third of great resistance. These mice were immunized once, twice,

or three times. The number of bacteria given at a dose was 1.25×10^7, 1.25×10^6 or 1.25×10^5. The whole design containing 27 treatments was completely balanced, equal numbers of mice being present at each treatment. After immunization and a lapse of 21 days the mice were injected with a rather massive dose of one of the bacterial strains, 5×10^7 organisms of 11 C. The results of these experiments bring out two interesting facts: 1. The three genetically different strains of mice show the same relative resistance to the typhoid organisms after immunization that they had prior to immunization. The dose necessary to bring about death, however, was about a hundred times greater after immunization than before. 2. The bacterial line of low genetic virulence was poor in immunizing capacity. The two virulent lines were both reasonably good immunizers. The results are shown in the succeeding table:

TABLE II

RELATIVE RATES OF SURVIVAL OF IMMUNIZED MICE AFTER INOCULATION WITH 5×10^7 ORGANISMS OF THE VIRULENT CULTURE

Immunizing Organism	Strain of mice			
	Ba	Z	RI	Mean
9 D	8.1	11.0	10.5	9.9
11 C	2.7	37.5	66.3	35.5
DSC1	5.8	30.9	60.5	32.4
Mean	5.6	26.5	45.8	25.9

CHARACTER BASIS FOR INHERITED HOST RESISTANCE

Our studies have shown that genetic resistance to mouse typhoid pathogen can be split up and the host differences segregated into pure breeding strains each characterized by a particular resistance. What inherited characters in the host are responsible for this resistance?

THE BLOOD CELLS

Our first study dealt with the characteristics of the blood cells. These studies showed that numbers of leucocytes were high in the highly resistant lines and low in the susceptible lines, the intermediate lines falling in between these extremes. The correlation between leucocyte numbers and resistance was high. The type of leucocyte did not seem to be of much importance; rather it was the total number. This suggests that the body can call out the type of leucocytes it needs to meet particular environmental circumstances. The numbers of leucocytes are an inherited character of the strains.

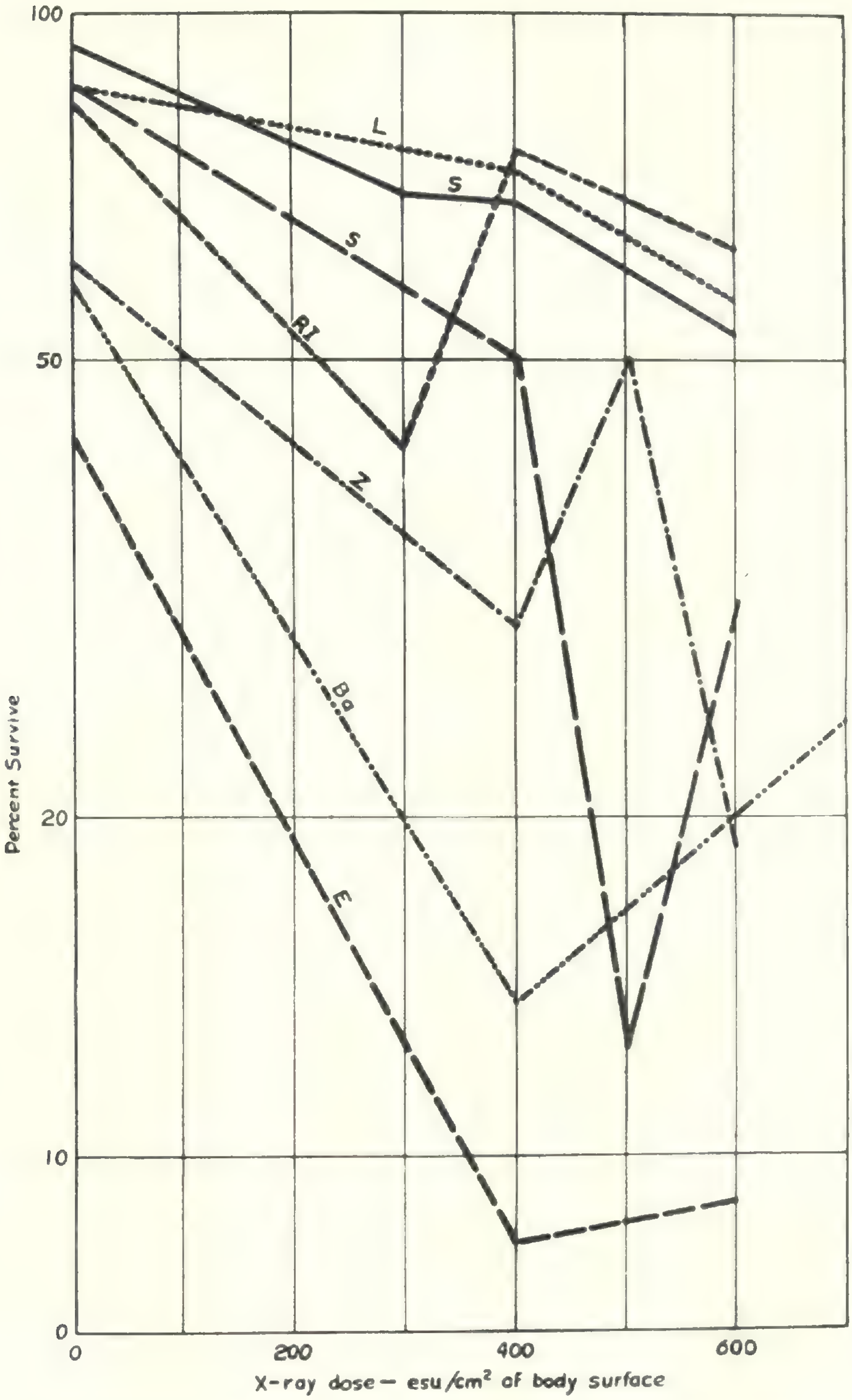

Fig. 6. X-ray treatment of different strains of mice as a means of reducing their resistance to mouse typhoid.

The erythrocyte numbers of the blood are also inherited and fixed for the different lines but the numbers fixed in the different strains have no correlation with leucocyte number or resistance. This is as we might expect if the erythrocytes play no part in the immunity.

X-ray exposure is known to modify the numbers of blood cells. Suitable use of X-rays should thus furnish further information on the part these cells play in the genetic resistance. Some 1256 mice have been treated with X-rays for these experiments; the dose ranged from 0 to 700 e.s.u. per square centimeter of body surface. With more than 700 e.s.u. the mice are so adversely affected as to show severe damage; above 1200 e.s.u. many mice die as a result of the X-ray treatment alone.

The mice were irradiated at about 52 days of age, then allowed a period of 8 days in which to recover from any immediate damage. At about 60 days of age, the S, RI, Z, and E strains were inoculated with 200,000 organisms of medium-virulent typhoid culture. The L and Ba strains were so susceptible that they were inoculated with but 100 organisms of the same culture. The results of this treatment are shown in fig. 6.

All seven mouse strains show a pronounced effect of the previous X-ray treatment on the capacity of the animal to survive inoculated mouse typhoid. The data are plotted as the log of the percentage of surviving mice against the X-ray dosage. Irregularities in individual observations occur but the over-all result is a uniform decline in survival as the X-ray dosage is increased. Between strains there is again a variation in the slope of this decline. This is to be expected on purely random grounds. Some measure of its possible significance can be had by comparing the two S strains as these two curves are really tests for but one strain.

It is evident from the plot that the effect of the X-rays on survival takes the form of the simple exponential equation:

$$\text{Survival} = ae^{bd}$$

Where *a* is a constant, *e*, the base of the natural logarithms, *b*, the term measuring the effectiveness of the X-rays, *d*, dose, measured in e.s.u. The effectiveness of the X-rays, *b*, for the two like tests on the same strain, S, is —.0009 and —.0023. The variations of our experiments are evidently such that a difference of this magnitude can be interpreted as due to uncontrolled causes. The constants for the X-ray effectiveness of all strains or the general slopes of the survival line are:

Strain	*Slope (b)*
S	—0.0009
S	—0.0023
RI	—0.0004
Z	—0.0015
E	—0.0028
L	—0.0007
Ba	—0.0014

It is evident that the slope constants are all within the same range. In fact, tests for significance of the differences show that the error within each strain .036 with 17 degrees of freedom is larger than the differences between regressions .027 with 6 degrees of freedom. We may therefore conclude that X-rays affect all strains in a similar manner.

This is important confirmatory evidence that the leucocytes are significant to the physical basis of genetic resistance to mouse typhoid. It is well known that X-rays destroy leucocytes. If the absorption of one unit of ray energy is sufficient to cause the destruction of a leucocyte or its primodial cell, then we would expect the leucocytes to decline according to the form, leucocytes $= ae^{bd}$, as the X-ray exposure, d, is increased. There is a linear relation between survival to typhoid and numbers of leucocytes. We should therefore expect that this decline would give comparable declines in the survival of the different strains to typhoid as the X-ray dosages increase. The data fit this view.

ORGAN AND CELLULAR DIFFERENTIATION IN DISEASE RESISTANCE

Clinical and cytological observations of mice which succumb to inoculations of 200,000 bacteria have shown that mice of the susceptible strain develop extensive lesions in the spleen and moderate ones in the liver. Mice of the resistant strain show no necrosis of the spleen and extensive destruction of the liver tissues.

Cytological studies of liver and spleen in inoculated animals show that with the onset of morbidity glycogen practically disappears from the livers of mice with low and intermediate resistance while glycogen storage is normal in the most resistant mice. With progress of the disease, susceptible mice show extreme fatty degeneration of the liver while the resistant strains show degeneration only as associated with the lesions. These observations indicate that the tissues of the resistant host are able to carry on their normal function even in the presence of the relatively large amounts of toxin which must be present to produce the severe hepatic necrosis which is characteristic of resistant strains.

Bacteria are visible in the liver and spleen about four days after inoculation. In the susceptible strains, bacteria always appear and usually continue to multiply until they kill the host. In mice of intermediate resistance the bacteria are present in the liver and spleen but in about half of the mice they disappear by the 8th to 11th day. The resistant mice, on the other hand, appear to destroy the bacteria rapidly for bacteria are never visible in the liver and spleen of most animals. When bacteria are present, they are usually found in definite lesions.

The spleens of the different strains appear to differ in the white pulp. Resistant strain shows more of that part of the organ than the susceptible strain. Such a difference should be directly associated with the number of macrophages per unit area of the spleen for these cells evidently arise from the lymphocytes of the white pulp both normally and during the progress of the disease.

The genetic capacity to resist or be susceptible to mouse typhoid evidently depends upon several different types of organ and cellular reaction. The particular

types are fixed within the strain by their genetic constitutions.

It might be thought that the humerol elements in the blood might also vary from strain to strain and play a part in disease resistance. Studies have been made on the agglutinative power and also on the bactericidal power of the serum of different strains.

AGGLUTININS AND DISEASE RESISTANCE

It is conceivable that the genetic selection and controlled breeding of the resistant lines could have led to fixation of natural agglutinins to *Salmonella* within these lines. If this were true, their immunological differences could be accounted for by such differences. Tests for natural agglutinins have been carried out on more than 100 mice of each strain but none have been found. Natural agglutinins seem to play no part in the genetic resistance observed in our strains of mice.

BACTERICIDAL POWER OF THE SERA

The natural bactericidal power of the blood could also have played a part in the genetic resistance. Tests for it in more than 60 mice of each strain have also shown it lacking.

GENERAL VIGOR AND DISEASE RESISTANCE

Since the days of Hippocrates it has been thought that some over-all element of disease resistance as general constitutional well-being played a definite part in resistance or susceptibility to many different diseases. While we have shown that such a general over-all condition does not seem to play any part in the resistance to unrelated diseases, it has seemed worth-while to examine the question for the typhoid organism.

Duration of life appears to be a good measure of vigor. A study was made of the duration of life of our six strains of mice. These studies show great differences in the length of life. Some strains are short-lived, others long-lived. Search for infectious causes of death have failed to reveal any of the common disease agents. At 60 days of age one could not pick out the long-lived strains from the short-lived strains by their appearance. In fact, in ordinary life, where internecine strife is a contributing cause of death between the males, one of the short-lived strains is a constant winner. The ability to survive is a clear-cut inherited difference.

This character has a high correlation with resistance to typhoid. The long-lived strains have high resistance; the short-lived are susceptible. Something in the genetic make-up of these long-lived strains favors resistance to typhoid even though previous contact with the organism has been wanting.

DISCUSSION AND SUMMARY

Before attempting an explanation of these results it may be wise to summarize them. For the pathogen a given organism may gain or lose virulence with equal

suddenness. The gain or loss of virulence may extend to very large populations if sufficient time elapses and the selection pressures are great enough. The changes are entirely comparable to mutations in the phenotype of higher forms. Bacteria or viruses may mutate to a multitude of various different types which later will breed true to the new type. These facts point to a relatively large number of genes, with capacities for variation within the pathogen. Some of these genes may mutate independently of virulence. The mutation of others may change the virulence type of the organism. This, too, would be expected from evidence on *Drosophila;* visible mutations may sometimes affect sterility whereas other mutations have no effect on sterility. There seem to be two rather distinct types of these variant changes. One of these has progeny showing only the variant type; the type is stable to the mutant type in the sense that most mutations in higher forms remain stable. Another type has progeny which show both the variant type and a new type. Progeny of the new type remain stable to the new type. Progeny of the variant continue to break up in successive generations to the variant type and to the stable type. These two types of variants, stable and unstable, occur with fair frequency. Temperature effects on the rate of change of the two types suggest that the mechanism behind the mutation processes may be different for each.

Mutations in tobacco mosaic virus show a pattern similar to that of bacteria. The mutations may or may not affect virulence. The different stable mutants observed from a given parent strain are frequently difficult or impossible to separate serologically from the parent strain. On the other hand, widely different virus strains may show three or four antigenic types. These facts suggest that tobacco mosaic virus, although possibly a single molecule, may have multiple antigenic properties, despite the fact that a mutant may not always be distinguished from its parent type in this respect.

These facts have important bearings on gene structure. They would seem to show that if a gene is likewise of molecular type it could have side chains, one responsible for one set of phenotypes and another for another set, the different sets seemingly affecting quite different processes. Such a model of the gene is not the one commonly drawn from the evidence on other forms. The tobacco mosaic units have a structure which suggests another possibility. The tobacco mosaic unit is seemingly built up of sub units, i. e. 2.2 mμ x 2 mμ x 2 mμ or perhaps more probably 37 mμ x 15 mμ x 15 mμ; the larger molecules being the multiples of this type, i. e. 300 mμ in length. The variant types could each be associated with a different sub unit, the whole being more like a chromosome in structure as Bawden has also suggested. The fact that the nucleic acid in each is of different type does not invalidate this parallelism but rather emphasizes the significance of the two models.

For an examination of how this variant behavior of the pathogen affects the host, we may turn either to the results on the domestic fowl and its typhoid organism, *Shigella gallinarum*, or to the mouse and its typhoid organism, *Salmonella*

typhimurium. The host, through genetic means, may be differentiated into pure-breeding strains each with characteristic resistance to a given line of the pathogen. In the mouse we have six such strains. The Ba and L strains have low resistance, the E and Z strains have medium resistance, and the S and RI strains have great resistance to the disease organism. The resistance differences appear due to the cellular pattern of the mouse as indicated by the blood, spleen, and liver. We have been unable to find evidence for the resistance differences being due to any humerol constituents as, for instance, agglutinins or bactericidins in the blood serum. General constitution as measured by normal duration of life is highly correlated with strain resistance to typhoid and may be a cause of the degree of resistance.

There is an interaction between the genetic constitutions of the pathogen and the host as shown by testing the different strains of mice with the different lines of bacteria. With living organisms the low virulent bacteria show a few deaths in the susceptible mice, a very few in the medium-resistant, and almost none in the resistant strain. With medium-virulent organisms there are a fair number of deaths in the susceptible mice, a lesser number in the medium-resistant mice, and a very few in the resistant mice. With the highly virulent line the deaths in the susceptible group are almost 100 per cent, are medium to high in the medium-resistant, and are low to medium in the resistant mice. If killed bacteria of the three lines are used, the same resistance levels are denoted in the different strains of mice but to get them it is necessary to use 100 times as many or more of killed organisms than of the living organisms. These facts show that the capacity for growth of the bacteria is not the only factor in this disease difference. Rather it indicates that there are different levels of endotoxin in the three lines of bacteria, each working on hosts of differing resistance. The mechanisms of these changes may be considered as follows: The three lines of bacteria may be regarded as of three distinct genotypes. Under the particular gene influence these lines develop an endotoxin within the bacterial cells. The endotoxin is such that it does not escape from the cell into the surrounding medium unless the cell itself is broken down. The endotoxin within each cell could be either different in amount for each line or different in type. As yet we have no evidence on this point. The living bacteria introduced into the host result in increasing amounts of endotoxin with the growth and death of the bacteria. The bacteria generate humerol antibodies, demonstrable through agglutination of the bacteria, in the host. These humerol antibodies probably take little part in the animal's immediate resistance for they appear too late in the course of the disease. They would, however, have the capacity to combine and neutralize a fair amount of toxin at a later date providing the animal survives. The main resistance mechanism so far analyzed appears to be the leucocytes of the blood and macrophages of the spleen and liver. These cells have affinity for the bacteria and for their endotoxins. The rapid disappearance of the bacteria in the resistant animals and the evident resistance shown in the formation of rather extensive lesions in some of the strains support this view.

Immunizing mice with killed bacteria prior to inoculation with living organisms indicates the mechanism by which artificial immunization may take place. In immunization the bacteria with the inheritance for low virulence have a low immunizing value. The bacteria for higher virulence have higher immunizing value. These facts point again to the genes in each of the bacterial lines as governing the formation of endotoxin either differing in amount or kind. On introduction into the host each bacterial line generates a characteristic amount of humerol antibodies in the host circulation. The cellular resistance of the host remains the same as or is increased somewhat over that of the unimmunized mice. When these immunized mice are inoculated with living bacteria of one of the different lines, it takes 100 or more times the number of bacteria to cause death as it would if the mice were unimmunized. The comparative death rates of the different strains of mice, however, remain as they were for the original untreated strains. The introduction of the dead bacteria into the host has made endotoxin characteristic of the particular line of bacteria. These endotoxins have resulted in the generation of humerol antibodies demonstrable through agglutination. These antibodies are of medium strength for the avirulent line of bacteria and of fair strength for the higher virulent lines. Presumably on introduction of the living organism these antibodies combine with them to agglutinate them and possibly to destroy some of the endotoxin through neutralization. The capacity for the resistance is thus increased about 100 fold. The cellular resistance mechanism likewise holds for the strain. Both the strains of mice and the lines of bacteria hold their relative positions in the immunization picture that they held in the case in which no immunization took place. It is simply that the levels of resistance are on a higher plane.

Genes differing possibly in side-chain structures, form H and O antigens and endotoxins differing in their capacities to produce agglutination or death. The antigens or endotoxins may reflect differences either in chemical structure of the genes as different antigens or endotoxins or, what now seems more likely, the capacities of the genes to produce small or large quantities of a single antigen or endotoxin. Host differences are attributable to host gene differences leading to the production of few or many of particular types of cells, i. e. macrophages; with specific capacities to destroy these bacteria or neutralize small or large amounts of endotoxin.

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GENE ACTION IN PARAMECIUM[1]

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I. INTRODUCTION

Gene action in *Paramecium aurelia* has been investigated (Sonneborn, '43a) most fully in the case of the action of the gene involved in the determination of the difference between "killers" and "sensitives." One race of this species makes the fluid in which it lives poisonous to nearly all other races of *Paramecium*. This race is thus a "killer." Killers are invariably resistant to their own poison. Races affected by this poison may be called either "sensitives" or "non-killers," for sensitives are never killers. Likewise, non-killers are never resistant. The killer character is determined by the combined presence of a dominant gene, K, and a cytoplasmic factor, kappa. Killers always have kappa; without kappa, clones are always sensitive non-killers. Killer clones also always have, in addition to kappa, gene K; but non-killers, lacking kappa, may have either K or its recessive allele, k. Thus, neither K nor k can initiate the production of kappa. Nevertheless, there is some relation between these genes and kappa, for clones containing kappa always have K and are never homozygous for k. The role of the genes is shown by observations on homozygous recessives (kk) produced by the self-fertilization of heterozygous killers (Kk plus kappa). In these recessives kappa is retained for a few fissions, during which the cells remain killers; hence, cells are killers when kappa is in the cytoplasm and K is absent from the new nuclei (though still present in disintegrating parts of the old nucleus). However, after a few fissions, kappa disappears and the clone becomes and remains permanently non-killer. Hence, kappa is not independently self-multiplying; it depends upon gene K for its maintenance and increase. The role of the genes is further shown by introducing kappa into non-killers. If the non-killer has the genotype kk, the resulting clone is still a non-killer; but if the non-killer has the genotype KK or Kk, introduction of kappa results in its maintenance and increase, yielding a clone of killers. Hence in relation to gene K, kappa acts something like a primer in a pump: some kappa is put in and more comes out. K seems to be like a pump that will not work without being primed. The action of gene K in controlling increase of kappa is thus dependent upon a cytoplasmic primer, kappa itself.

The work just summarized suggests that an understanding of the action of gene K might be acquired by a detailed investigation of its relations to kappa. An investigation of these relations is reported in a paper now in press (Sonneborn,

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[1] Contribution No. 345 from the Department of Zoology, Indiana University.

[2] The Rockefeller Foundation and Indiana University contributed to the support of the researches on which this paper is based. Miss Ruth V. Dippell gave invaluable assistance in the conduct of the experiments.

'45), in which a hypothesis of gene structure and action is induced from the results of the experimental analysis. The main features of this hypothesis, however, were suggested in the first place by the results summarized above and certain other facts known much earlier; the experimental analysis was in fact designed specifically to test the hypothesis. I shall therefore take this opportunity to point out how the hypothesis was indicated by the available information and how it was put to experimental test. This will make it possible to put on record pertinent material not presented in the other paper.

II. INFORMATION LEADING TO A HYPOTHESIS OF GENE STRUCTURE AND ACTION

The hypothesis was suggested in the first place by an attempt to account for the observed striking difference between the gross genetic phenomena in different varieties of *Paramecium aurelia*. In variety 4, all characters (killers, mating types, antigens) studied show essentially the same system of determination and inheritance. In every case, a cytoplasmic factor intervenes between gene and character. The gene alone cannot initiate production of this factor, though maintenance and increase of the factor are under the control of the gene. On the other hand, the characters (mating types, antigens) studied in variety 1 are determined by genes whose action does not depend upon cytoplasmic primers. Of the other varieties of *P. aurelia* thus far studied, some show the system characteristic of variety 1; the rest show the system characteristic of variety 4.

Two reasons make it appear unlikely that this difference between the genetic phenomena in the two groups of varieties could be due to any very profound difference in the genes and their mode of action. One reason is the extremely close relationship of the varieties; the other is the apparent identity in the two groups of varieties of the cytological processes on which the genetic phenomena must depend.

The seven varieties of *P. aurelia* thus far discovered differ little, if at all, morphologically. They are so much alike that they would never have been recognized without the discovery of mating types. They are, in fact, defined simply on the basis of sexual isolation. Within each variety, every individual belongs to one or the other of two mating types which interbreed freely; but neither mating type in one variety interbreeds with either mating type in any other variety. (Recently exceptions to this rule have been discovered, but they do not essentially alter the effectual isolation of the varieties.) Clearly, the diverse varieties are so closely related that they could hardly have diverged appreciably in anything so fundamental as gene structure and action.

Likewise, the visible cytological features of the fertilization processes are identical in the different varieties. In all varieties the diploid micronuclei in each conjugant undergo two meiotic divisions and all the resulting haploid nuclei disintegrate except one. This one goes through a third (equational) division, the two products of which are the gamete nuclei. One gamete nucleus in each con-

jugant passes into the mate and fuses with the gamete nucleus that has remained in the mate. This fertilization nucleus, in which the diploid condition has been restored, gives rise by ordinary equational mitoses to diploid nuclei that develop into the new macronuclei and micronuclei. Meanwhile, the macronucleus originally present in each conjugant disintegrates into many pieces which are eventually resorbed. In the process of exchange of gamete nuclei no cytoplasm (or effectively none) is normally exchanged. Conjugation thus provides for an exchange of genes, but no cytoplasm; and the resulting genotype of the two mates must be identical because each of the two haploid nuclei that unite to form the syncaryon in one conjugant has its exact copy in one of the two that unite in its mate. The cytological features of conjugation thus provide no basis for the observed difference in genetic phenomena among the varieties.

Nevertheless, as set forth above, exchange of genes alone brings about identity of characters in the two mates in variety 1 and not in variety 4. In the latter variety, identity of characters develops only in those exceptional matings in which both genes and cytoplasm are exchanged. It would seem as if the action of a gene in variety 1 must be equivalent to the action of a gene plus its cytoplasmic factor in variety 4. This relation is emphasized by the fact that entirely comparable characters, such as mating types or antigens, depend upon a gene only in variety 1 and upon a gene plus a cytoplasmic factor in variety 4. Further, in variety 4 different characters depend upon different cytoplasmic factors: each cytoplasmic factor is related to a particular gene. It seems improbable that a character, such as an antigen or a mating type, could in closely related varieties be the result of biochemical materials and processes so diverse that, in one variety, a gene alone is capable of controlling them while, in another variety, the gene requires in addition a cytoplasmic primer. These reflections forced me to consider the possibility that each gene in variety 1 includes what is distributed in variety 4 between a gene and a cytoplasmic primer. In other words, the cytoplasmic primers in variety 4 may correspond to a *part* of the gene in variety 1.

This possibility is obviously in conflict with what has been regarded as a fundamental conception in genetics: the indivisibility of the gene (see, e. g., Wright, '41). Is there any reason why separable parts of the gene might be discoverable in *Paramecium* and not in other kinds of organisms? The answer, it seems to me, is provided by consideration of the peculiar nuclear conditions characteristic of the ciliated Protozoa. Only in these organisms does there exist a distinction within each cell between a physiologically functional and a physiologically non-functional nucleus. The macronucleus is indispensable and controls the physiological activities of the cell; but the micronucleus is not essential: clones live and multiply well without it and maintain their genetic characters. Consequently, loss of physiologically important parts of the micronuclear genes would be of no importance to the cell. Hence the nuclear conditions in the ciliated Protozoa are such that loss of physiologically active parts of the micronuclear genes is a theoretical possibility. In other kinds of organisms such special nuclear

conditions do not exist and there is consequently no opportunity for gene disintegration to occur with impunity and so to be capable of detection.

By the same reasoning, however, one may conclude that the physiologically active macronucleus should in general retain the complete genes, for disintegration of these would be fatal to the organism. It would seem, therefore, that no essential difference should exist between the macronuclear genes in different varieties of *P. aurelia*. If the cytoplasmic primer in variety 4 corresponds to a part of the gene in variety 1, it should exist as a part of the macronuclear genes in both varieties.

This leads at once to the question of how the macronuclear genes could acquire their primer parts. There is no difficulty in variety 1 because both parts of the gene occur in the micronuclei which transform directly into macronuclei after fertilization. In variety 4, on the other hand, the micronuclei that give rise to macronuclei lack the primers. Previous experiments (Sonneborn, '43a) have shown, however, that the primers are in the cytoplasm at the time of fertilization. The new macronuclei at the time of their origin are therefore surrounded by cytoplasm containing the primers and could obtain them from this source. Further, the old macronucleus always disintegrates prior to the formation of new macronuclei, thus providing the cytoplasm with primers for the latter. The visible cytological processes accompanying fertilization thus supply a mechanism for the transfer of primers from macronucleus to macronucleus in those varieties in which the micronuclear source is cut off.

Here then is a clue to a mechanism of priming in variety 4. Gene K is the micronuclear gene which is normally transferred through the gamete nuclei at conjugation. In this form it is self-multiplying, both in micronucleus and macronucleus. If it unites, in the macronucleus, with kappa obtained from the cytoplasm, the complete gene is constituted—comparable to the micronuclear genes in variety 1—and undergoes self-duplication in the complete form. The necessity for priming arises from the separation of the parts of the gene and the inability of one part of the gene to produce the other part. In effect, K and K plus kappa are alleles and the change from one to the other is a mutation. The primary action of a gene, on this view, is self-duplication: K controls the production of kappa by reason of the fact that kappa becomes a part of the gene and thereby is reproduced as a part of it.

III. TESTS OF THE HYPOTHESIS

The preceding considerations seemed to justify the adoption, as a working hypothesis, of the assumption that K and kappa are capable of union and are in fact united in the macronucleus of killers. This hypothesis was subjected to the experimental tests presently to be set forth. To understand the tests, however, it is necessary first to recall certain important features of the macronucleus.

As set forth above, the macronucleus arises from the syncaryon as a simple diploid nucleus. It then grows enormously, becoming a multiple nucleus containing at least 30 units, each with a complete diploid set of genes. At each fission

the macronucleus divides amitotically, approximately half of the component unit nuclei passing to each daughter nucleus. However, the unit nuclei themselves must (on the basis of genetic evidence) divide by some sort of mitotic process, though the two products of division of a unit probably pass as a rule to the same daughter macronucleus. At times of fertilization, the compound macronucleus falls apart into its component units and these are resorbed in the cytoplasm.

With these features of the macronucleus in mind, the hypothesis that K and kappa are combined in the macronucleus could be tested if there were available a method of varying the amount of kappa introduced into non-killers containing gene K. For the K genes of the macronucleus may be considered as specific receptors for kappa and, if small enough amounts of kappa are presented to a macronucleus containing many K genes, there should not be enough to combine with all of these genes. Consequently, the amitotic divisions of the macronucleus during the course of repeated fissions would have to yield eventually some macronuclei completely devoid of kappa and some completely saturated with kappa. The former therefore could not yield killers, while the latter could.

The required method was developed by taking advantage of the following observation. When killers are crossed to non-killers, normally no cytoplasm (or effectively none) is exchanged and the conjugant pairs separate quickly after fertilization is completed: less than 3½ minutes elapse from the beginning of the separation process (at the anterior ends) until it is completed (in the region of the peroral cones across which the migratory gamete nuclei pass during fertilization). In the exceptional cases in which more than 30 minutes is involved from the beginning to the end of the separation process, cytoplasm is invariably exchanged and in amounts sufficient promptly to transform the non-killer mate into a killer. When the separation process takes an intermediate time, intermediate results are obtained presumably because intermediate amounts of kappa pass from the killer to the non-killer mate. This then provides a method of introducing reduced amounts of kappa into KK non-killers. However, the kappa is introduced at the time of fertilization and therefore before the syncaryon has produced the simple diploid nuclei from which the new macronuclei are to arise. Kappa is consequently present in the cell at the time the presumptive new macronuclei are in the simple diploid condition. Entrance of kappa into them at this time would result in macronuclei saturated with kappa. The results now to be set forth, however, indicate that the macronuclei may fail to become saturated. It appears, therefore, that kappa, when present in the cytoplasm in small amounts, does not necessarily get to the K genes of the new macronucleus before the latter begins to acquire its multiple condition. For the clones developed from such conjugants showed precisely the predicted segregation of kappa during the course of vegetative reproduction: lines of descent totally lacking kappa arose within these clones after from one to nearly 90 successive fissions, in different instances. Kappa, then, is unequally divided at fission in agreement with the amitotic division of the macronucleus.

While the preceding result was predicted on the basis of the hypothesis, the character of the lines which retained kappa (and the great majority did) was totally unexpected: they failed to become killers during long periods of asexual reproduction. That kappa was being produced and maintained all this time was nevertheless clearly demonstrated both by the ability of these non-killers to transmit kappa to other cells during conjugations involving cytoplasmic exchanges and by the fact that they usually yielded 100 per cent killer progeny when they underwent self-fertilization.

The 100 per cent maintenance of the non-killer character during vegetative reproduction and the 100 per cent transformation into killers after fertilization, while not foreseen and predicted, finds a simple explanation on the hypothesis under analysis. It is in fact precisely what would be required on this hypothesis, if the kappa combined with K in the macronucleus is unable to get back into the cytoplasm from the intact macronucleus, and if the killer character depends upon the presence of kappa in the cytoplasm. Under such conditions the non-killer character of the lines that maintain kappa is due simply to the combination of all the available kappa with K genes in the macronucleus leaving none (or effectively none) for the cytoplasm. The transformation of these non-killers into killers after fertilization would follow from the great excess of the kappa released into the cytoplasm at the time the compound macronucleus disintegrates, over the relatively small amount needed to saturate the K genes of the simple diploid or slightly compound macronuclei when they first develop after fertilization. As a consequence of the great disparity between the amount released by the many K genes of the old macronucleus and the amount with which the few K genes in the new macronuclei can combine, much is left over for the cytoplasm and the cell gives rise to a killer clone.

The observations on the consequences of introducing very small amounts of kappa into KK non-killer cells that previously lacked kappa are thus in agreement with the proposed hypothesis that K and kappa are united in the macronucleus when both are present in a cell. The observations have further indicated (1) that the kappa thus combined with K in the macronucleus does not escape from the intact macronucleus into the cytoplasm; and (2) that the phenotypic action of kappa depends on its being present in the cytoplasm. These ideas could be tested in another way if there were a method by which the number of K genes could be greatly increased without a corresponding increase in the amount of kappa. For this should lead by a different route to the same result as that obtained in the preceding experiments: in both cases the situation would be such as to yield some K genes lacking kappa.

This type of experiment is possible by taking advantage of the following known facts. At the time of fertilization, the old macronucleus, as has been said, breaks down into 30 or more pieces each of which contains at least one full diploid set of genes. Furthermore, the preceding experiments showed that KK non-killers containing kappa have kappa in the cytoplasm at this time. If, as the

experiments indicated, the kappa was previously combined with K in the macronucleus, the kappa must be released into the cytoplasm when the macronucleus disintegrates into pieces. During the course of the next two cell divisions, the new macronuclei which have arisen from the syncaryon presumably take up kappa. While the new macronuclei are growing and developing their normal compound condition, the pieces of the old macronucleus also grow though they are destined soon to be resorbed in the cell. This growth of the pieces of the old macronucleus involves at least a four-fold increase in volume during these first two cell divisions. This suggests that the genes of the macronuclear pieces undergo some multiplication after they have released kappa and before they are resorbed. At this stage, it is possible experimentally to suppress the division of the new macronuclei so that at the second postzygotic cell division some of the cells fail to get macronuclei. The pieces of the old macronucleus, however, are passively distributed (without division) among the daughter cells, so that each of the four cells gets about one-fourth of the pieces. In the cells that lack macronuclei but possess pieces of the old macronucleus, the latter not only fail to be resorbed, but each piece regenerates into a complete compound macronucleus (Sonneborn, '40, '42). The pieces are distributed at random during subsequent fissions until there is only one per cell, and thereafter this one, which by that time has reached full macronuclear size, divides normally at subsequent fissions. In this way, therefore, it is possible to get the many K genes of the pieces of the old macronucleus and the K genes they produced after they lost kappa to become functional and hence again to be receptors for kappa. In other words, many K genes normally destined to be lost are retained and multiplied and so provide a greatly increased number of kappa receptors.

This technique of increasing the number of receptor K genes was applied to animals of the pure killer race 51 in which non-killers had never before been found, in order to discover whether the increase in number of K receptors for kappa would yield any K genes lacking kappa. As in the previous experiments, macronuclear amitosis should eventually yield lines lacking kappa if any of the K genes of any of the regenerated macronuclei lacked kappa. The experiment did in fact yield both non-killers containing kappa (as in former experiments with introducing small amounts of kappa into KK non-killers) and non-killers from which kappa disappeared permanently, never reappearing in the course of subsequent vegetative or sexual reproduction. The experiments involving increase of K in killers thus leads to the same results as introducing small amounts of kappa into KK non-killers; both types of experiments yield the results predicted on the hypothesis that kappa is a dissociable part of gene K.

IV. DISCUSSION

A. *Darlington's views on the action of genes K and k.*—Darlington ('44) has suggested that kappa is not maintained by K, but is either inactivated by k

or is overgrown by a competitive plasmagene controlled by k. It has been shown above, however, that a pure killer race can be induced to yield a pure non-killer branch from which kappa permanently disappears without the introduction of k or any product of k into the organisms. This result renders Darlington's suggestion unnecessary and unlikely; and the means employed in the experiment to bring about the result support the alternative interpretation that K controls the increase of kappa. In a previous paper (Sonneborn, '43b) loss of killing action was reported in another race (47), and Darlington attributed this to hybridization with a race containing k. However, race 47 was isolated from all other races in the laboratory and had never been hybridized with another race during the years it was a killer or for a long time after it had become a non-killer. Nor has it at any time given any indication of containing gene k. In the absence of any evidence for Darlington's suggestion as to the role of k and, particularly, in view of the contrary evidence for the action of gene K, his interpretation seems unacceptable.

B. *Some unsolved problems.*—The major problem that remains unsolved is to account for the increase of cytoplasmic kappa. The material in sections II and III provides an explanation of how kappa primes K to produce more kappa in the macronucleus; but no data and no suggestions have yet been given as to how kappa is multiplied in the cytoplasm. It is known only that increase of kappa in the cytoplasm (which must occur in killer clones) depends upon the presence of K (and kappa?) in the macronucleus. The simplest suggestion appears to be that increase of cytoplasmic kappa may be brought about in essentially the same way as is the increase of macronuclear kappa, i. e., by combination with K, a part of K, or a product of K which, unlike kappa, is capable both of passage from the intact macronucleus to the cytoplasm and of very limited self-duplication in the cytoplasm. On this, however, there is as yet no experimental evidence.

Many other questions also need to be answered. Do the genes of variety 1 contain primers, as the hypothesis holds? Can alleles differ in the primer component only, i. e., can more than one kind of primer combine with the same basic part of the gene? If so, can two or more kinds of primers combine simultaneously with the same basic part? Is the primer alone the active part of the gene, or is the physiological specificity of the gene partly dependent on the basic gene also? These questions and others are now under investigation.

C. *Gene structure and action.*—The present paper presents and tests the hypothesis that the gene consists of two parts. One part, K in the example analyzed, is found in the chromosome, though its occurrence or the occurrence of part of it in the cytoplasm has not been excluded; the other part, kappa, occurs in both the chromosome and the cytoplasm. K can exist and multiply without kappa, but kappa cannot long exist or multiply without K. K alone produces no detected phenotypic effect; but kappa determines the killer phenotype. The possibility still remains that K not only controls the multiplication of kappa, but also, when in the presence of kappa, plays a part in the determination of the phenotype. The main features of gene action appear to be (a) self-duplication, and

(b) providing the cytoplasm with replicas of itself or part of itself. No effect of the gene on the phenotype is detected when the complete gene is confined to the macronucleus; only when at least kappa is also in the cytoplasm is phenotypic action discernible.

That the gene may consist of two diverse parts was suggested by Correns ('19) to account for variegation and by Thompson ('25, '31) to account for the Bar phenomena. In neither case was it possible to follow separately the assumed two parts of the gene, and the hypotheses were in the main formal and not subject to experimental test. That a primary action of the gene is the liberation into the cytoplasm of complete or partial copies has been suggested by Koltzoff ('35), Wright ('41) and others. This view, although based on weighty general considerations, has also not been subjected to experimental tests.

The work here reported on *Paramecium* supplies experimental tests indicating both the bipartite structure of the gene and the identity between an active cytoplasmic component and a part of the gene. The data, especially when they are presented in detail, need to be critically examined to see if there can be suggested some satisfactory alternative to the conclusion that K combines with kappa in the macronucleus. I have been unable to find one, but others may succeed where I have failed. In the absence of such an alternative, the present work may serve as a beginning of an experimental approach to the difficult problems of the structure and primary action of the gene.

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SPONTANEOUS MUTATIONS OF BACTERIA

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The study of bacterial mutability is our only avenue of approach to problems of heredity in bacteria. This approach may be followed along two lines. Along one line one focuses his attention on the phenotypic differences between two strains which differ by one mutational step, in the endeavor to trace the reaction chains from the phenotype back to the gene. This is the analogue to physiological genetics and is exemplified, for instance, by E. H. Anderson's (1) comparative study of the physiology of virus-resistant bacterial mutants.

Along the other line one focuses his attention not on the mutant and its phenotype but on the mutational step, specifically on the rate at which it occurs under some standard conditions. At first sight such a study seems rather aimless because the use of determining this or that mutation rate is not apparent. Studies of this kind can, however, become of interest in two ways. It may be found that the mutation rate can be controlled by cultural conditions. Such a finding might give us a lead as to the nature of the mutational process. Investigations of this sort have not yet been reported in the literature but very likely could be undertaken with great profit. Another use of the study of mutation rates lies in the possibility of considering the mutational pattern of a strain of bacteria and of its mutants. Attempts along these lines have been reported by Luria (2), by E. H. Anderson (1), and by Demerec and Fano (3). It is quite evident from these papers that the same mutational step can be identified in organisms which differ by one or more mutational steps of a different kind. This use of mutation rates, and of mutational patterns, may prove to be an invaluable substitute for Mendel's experiments for purposes of factor analysis in bacteria.

The main problems which face the experimenter along this line are:

1. The estimation of the number of mutants in a given culture.
2. The evaluation of the mutation rate.
3. The differentiation between mutations which may lead to phenotypically similar forms, and conversely the identification of the same mutation if it occurs repeatedly.

Before discussing these topics, I would like to make a few general remarks about methods of detecting and culturing mutants of bacteria. Some of these remarks are also pertinent to the study of mutations of viruses.

1. POPULATION DYNAMICS AND MUTATION PATTERNS

We will assume that we start from a stock strain with fairly constant properties. Actually, a variety of mutations will occur during the subculture of such

a strain. The strain will, therefore, not be pure in the genetic sense, but will represent a population which is in approximate equilibrium with its mutants. The equilibrium will be determined by three factors, namely, the forward mutation rates, a_1, a_2, etc., which lead away from the normal type, the reverse mutation rates, b_1, b_2, etc., and the selection rates, s_1, s_2, etc.[1]

We will measure mutation rates in units of mutations per bacterium and per time unit, and selection rates by the difference between the growth rate constants[2], k_N, of the normal type, and k_M, of the mutant type,

$$s = k_N - k_M$$

The populational equilibrium between the normal type and its mutants will depend on the cultural conditions. If the cultural conditions are altered the equilibrium will in general shift, and the shift may or may not be reversible when the strain is returned to the standard conditions.

Certain conditions must be fulfilled by the mutation and selection rates for the equilibrium to be reasonably stable. For instance, if a certain forward mutation rate, a_1, is high, it must be balanced either by a high selection pressure against this mutant, or by a high reverse mutation rate.

If the mutant is balanced by adverse selection then the equilibrium proportion of mutants is given by[2]

$$M/(M + N) = a/s$$

and this fraction must be small compared to unity. If a sub-culture is started from a single normal bacterium, equilibrium will be approached with a rate equal to $s - a$. Since the mutation rate must be small compared to the selection rate, the approach to equilibrium is essentially determined by the selection rate.

If the mutant is balanced by reverse mutation then the equilibrium proportion of mutants is given by

$$M/N = a/b$$

Since this ratio must again be small, the reverse rate must be large compared to the forward rate. The rate of approach to equilibrium in this case is determined by the sum of the forward and reverse mutation rates, $a + b$, that is, essentially by b.

If the forward mutation rate is very small it need not be balanced by selection pressure against it or by reverse mutations because the mutant type will in general be eliminated on each sub-culture when small samples are used for the sub-culture.

It may sound strange that the conditions of equilibrium should differ for small and high mutation rates. Actually, of course, there is no difference in the

[1] Strictly speaking, we should also include among the factors which determine the population equilibrium all the rates of mutation of the mutants. However, most of these second-step mutations will cause only small shifts of the equilibrium. If a particular step should be fast the two steps can be lumped together as one mutation.

[2] See Appendix I at the end of this paper for definition of growth-rate constants and for mathematical derivations.

equations which determine the average values of mutants in a very large number of cultures in these two cases. However, for small mutation rates the fluctuations in these numbers become the dominant feature. Suppose we are dealing with a mutation rate of 10^{-8}. If we were dealing with millions of similar cultures, or if we would sub-culture one culture many million times we would be likely at one time or another to transfer a great many mutants, and from then on the culture would contain a high proportion of mutants. In actual experiments, however, we are not likely to encounter these rare events which are included in the ideal average.

When studying the mutational pattern of a stock strain with reasonably stable properties we may therefore expect to find, on the one hand, mutants which occur with high frequency but grow more slowly than the normal type or revert quickly, and, on the other hand, mutants which occur with low frequency and which may or may not be at a selective disadvaneage due to slow growth or reverse mutations. These expectations are borne out by the general experience that stable and vigorous mutants of stock strains always occur with very low frequency.

The same is not true of the mutational pattern of the mutants themselves. The only prediction that can be made regarding the patterns of the mutants is that mutants which occur frequently and are not at a selective disadvantage must have a high reverse rate. Such mutants are, of course, of great importance for the study of the mutational pattern of the normal type, but it stands to reason that their study presents special difficulties.

2. THE ESTIMATION OF THE NUMBER OF MUTANTS

We may distinguish between three methods of detecting mutants in cultures of bacteria.

The first method consists in plating out a sample of the normal culture so as to obtain isolated colonies. The mutants may then be picked out either simply by inspection of the colonies or by special tests of a large number of colonies selected at random. The first alternative is used for picking mutants which affect the morphology or pigmentation of colonies. Using the technique of Spiegelman, Lindegren and Hedgecock (4), it may also be found possible to pick by this method mutants which affect the fermentative properties of the strain. The second alternative has been applied by Roepke, Libby and Small (5) to obtain growthfactor-deficient mutants.

The second method is to subject the normal culture to a destructive treatment, such as irradiation with ultra-violet light (Maisel-Witkin unpublished) or exposure to penicillin (Demerec unpublished). If the normal population contains mutants with somewhat heightened resistance to these destructive agents, then these resistant mutants will be present in higher proportion in the fraction of bacteria which survives the treatment. In the ideal case the surviving fraction of bacteria will consist entirely of mutants with increased resistance. The method

amounts, in such a case, to a direct isolation of the mutants, but of course not all mutants with increased resistance present in the original culture are isolated by this method, because most of them will be killed by the treatment. Consequently, it is quite difficult to determine the fraction of this mutant type in the normal culture.

The third method is to use a treatment which will eliminate all normal bacteria while leaving all individuals of certain mutant types unaffected. The only treatment known that will give such a clear-cut segregation of certain mutant types is exposure to an excess of virus particles which are active on the normal strain of bacteria. Luria has shown that a similar principle can be used to isolate mutants of *viruses*, by plating a large number of virus particles with a strain of bacteria resistant to the normal type of virus of the stock used. Such stocks may contain mutant viruses which will attack and multiply at the expense of the bacterial strain.

Mutants must fulfill certain conditions to permit their maintenance and study. In general, reverse mutation rates must not be so high that the normal populational equilibrium is reached in a single sub-culture. In growth factor mutants the conditions are more stringent because even a very low reverse mutation rate will invalidate the test of mutation.

In virus-resistant mutants the conditions for maintenance are less stringent. Here the reverse mutants are automatically eliminated if plating is done in the presence of excess virus. If the reverse rate is rather high the mutant may become extinct in colonies starting from a single resistant bacterium. The chance for this to occur is $b/(1 - b)$, where b is the reverse rate per generation. The proof for this statement is given in Appendix II.

3. MUTATION RATES

In the past there has been much confusion about the concept of mutation rate. Many authors have simply divided the number of mutant bacteria found in a culture into the total number of bacteria and have called this fraction the mutation rate. It is obvious that the fraction of mutants is a poor measure of the mutation rate, since the number of mutants depends on two factors, namely, the number of mutations occurring in the culture and when they occurred. A mutation which appeared several generations back will be represented, at the time of the counting, not by one mutant, but by a sizeable clone of mutants.

First of all, we must define a mutation rate in such a fashion that it becomes a characteristic of each bacterium and expresses a property of this bacterium which can be measured (6). We define the mutation rate, therefore, as the probability of a bacterium to mutate during a given time unit under some specified physiological conditions. The time unit, of course, must not be greater than the lifetime of the bacterium. This conception of a mutation rate is quite analogous to the concept of probability of decay of a radio-active atom during a time unit.

In a growing culture of bacteria, mutations will occur with a frequency per time unit which increases in proportion with the size of the population. At any arbitrary moment, therefore, various-sized clones of mutants will be present in the population. Compare, for instance, clones of size 64 with those of size 128. Clones of these sizes originated in mutations which occurred respectively 6 or 7 generations back. Six generations back twice as many normal bacteria were present as seven generations ago. Clones of size 64 should therefore be twice as frequent as those of size 128. On the other hand, the average number of *mutants* belonging to clones of size 64 should just equal that belonging to clones of size 128. By logical extension one finds that the average number of mutants in a culture, say, 30 generations old, should fall into 30 groups of equal size, each group containing mutants of the same clone size. This statement, while correct, is not applicable to any real experimental situation, because the large clones, which contribute a large portion to the average, are so rare that they will not likely be found in a limited number of trials. The likely average number of mutants in a culture must therefore be calculated with omission of the contributions from the large but rare clones.

The likely average fraction of mutants thus turns out to be smaller than the ideal average fraction. The ideal average fraction would be equal to the rate multiplied by the time since the start of the culture. The likely average, on the other hand, is obtained by measuring the time, not from the start of the culture but from the time when the culture had reached a size at which a mutation becomes likely. Thus, for a mutation rate of, say, 10^{-6} per generation, we should count the time from the moment that the culture has reached a population size of about 10^6. If our observation is made when the population has reached a total number of 10^9, the time to be used would be 10, instead of 30, the number of generations since the start of the culture. Our correction in this case amounts to a factor of three. If the mutant grows more slowly than the normal type we must use a further correction to take into account that the mutants have not multiplied at the same rate as the normals.

The determination of the mutation rate from the number of mutants present at any given time suffers from another complication, namely, the very large fluctuations of the number of mutant bacteria in a series of similar cultures. It is easy to see why the fluctuations must be large. As we have seen above, the mutant bacteria, on the average, stem in equal numbers from all preceding generations. If the mutation rate is small, mutations will occur with any reasonable probability only during the last few generations, say the last ten generations as in the example above. Now the quantity which is subject to normal fluctuations is not the number of mutants but the number of mutations, in each generation. In the example cited one-tenth of the mutants will, on the average, be due to one mutation which occurred ten generations back, another tenth will be due to two mutations which occurred nine generations back, etc. It is clear that the one and two, etc., mutations are subject to large fluctuations. The net fluctuation in the final

number of mutants is therefore very appreciable, even if the number of mutants is quite large.

To make matters worse, the customary recourse to using large numbers of similar cultures for determining the average number of mutants is here of little use, because the more cultures we use the more likely we are to have a mutation occurring at a very early stage in the development of one of the cultures. Such an event is like hitting the jackpot; it will give us an erratic number of mutants and a most undesirable increase in the fluctuations.

To sum up this matter, we may say that the determination of the mutation rate from the number of mutants, even if we can measure the number accurately, is necessarily a statistically inefficient procedure.

4. REVERSE MUTATIONS

I should like to add a few remarks about the problem of studying reverse mutations. As has been pointed out before, reverse mutations, even if they occur frequently, do not interfere with the detection of forward mutations in mutations which affect the reaction of bacteria to viruses. While this is an advantage to the study of the forward rate it is a decided disadvantage in determining the reverse rate. At present we do not know of any generally applicable method for the determination of mutation rates from virus resistance to virus sensitivity.

Just the opposite of what has just been said is true of mutations which involve the acquisition of growth-factor requirements. These can be discovered only if the reverse rate is exceedingly small. However, if the reverse rate is small enough to permit the cultivation of the forward mutant then the reverse rate, even though it be very small, can be measured with reasonable accuracy. This is quite apparent from the work of Roepke, Libby and Small (5). Their method of detection of reverse mutants is to culture the factor requiring mutant in a basal medium to which a sub-optimal amount of the growth factor has been added. If reverse mutation occurs it shows by delayed full growth in this medium.

Now the mutants selected by any of the methods mentioned may differ from the normal in more characters than the one used to select it. Specifically, E. H. Anderson (1) has shown that many of the virus-resistant mutants are also mutants deficient in growth factors. In these special cases, therefore, the two methods might advantageously be combined; that is, one may use the virus resistance for the isolation of the forward mutants and the growth factor requirements for the detection of reverse mutation.

5. IDENTIFICATION AND DIFFERENTIATION OF MUTANTS

The chief interest of a study of a hypothetical pair of forward and reverse mutations would lie in ascertaining whether or not true reverse mutations do occur. It is difficult to decide whether the revert strain is identical with the original one. Theoretically, this implies the comparison of the original with the presumed revert strain in all measurable characteristics. In practice one has to

restrict himself to a selection of some fairly comprehensive tests. Tests with viruses are among the easiest.

The following example may serve as an illustration of the method. Two mutant strains of a bacterial strain (*E. coli* "B") had been isolated, one by using virus T1 as selecting agent, the other by using T5. These strains may be referred to as B/1 and B/5, respectively. Each of these strains was then plated with seven different viruses, T1 to T7, and with each virus they were tested in two ways, once by plating a large number of the bacteria (about 10^8) with an excess of virus particles, and once by plating with about 100 virus particles. The results are given in Tables I and II.

TABLE I

PLAQUES OBTAINED WHEN PLATING LOW TITER STOCKS OF VIRUSES ON B/1 AND B/5

	T1	T2	T3	T4	T5	T6	T7
B/1	0	183	118	146	0	47	59
B/5	0	160	106	172	0	44	64

Table I gives a comparison of the plaque counts obtained with these two bacterial strains for the seven viruses. It will be seen that both strains are resistant to both T1 and T5, and that they give equal plaque counts with all the other viruses. The two strains, therefore, agree in their resistance pattern.

At this point, however, we must inquire somewhat more closely into the nature of the mutations which make bacterial strains resistant to one or another group of viruses. It used to be believed that these mutations fall into two classes: On the one hand, there were thought to be mutations which completely alter the sensitivity pattern of the bacterial strain, and which in general also alter the morphology or other characteristics of the colony of the strain. These are the well-known "dissociative" changes from smooth to rough, etc. On the other hand, there were, it was believed, highly specific changes towards resistance to only one, or to a group of closely related viruses. These conceptions are due chiefly to Burnet (7), and the idea of classifying viruses by cross-resistance tests goes back to an important paper by Bail (8).

However, recent work has indicated that these conceptions are not tenable (9). There is no clear-cut distinction between mutations with generalized effects and those which cause nothing but resistance to a group of similar viruses. Specifically, it has been found that the classification of viruses by cross-resistance tests does not at all lead to a satisfactory grouping. Such classifications bring together viruses which are quite unrelated on other criteria, and they separate viruses which, on all other criteria, are most intimately related. The most important among these other criteria are the morphology as revealed by the electron microscope and cross-inactivation tests with specific antisera.

On second thought, we should not be surprised by these findings. Once it is granted that the mutations in question are not induced by the virus but are spontaneous, it seems understandable that one mutation may affect the sensitivity of the bacterium to quite unrelated viruses. At present, we do not know what makes a bacterium sensitive or resistant to a virus. We can imagine that certain substances must be elaborated whose presence permits the virus to grow in the bacterial cell. It stands to reason that a variety of genetic changes could interfere with the network of synthetic processes in such a fashion that the bacterium becomes unsuitable for the growth of the virus. Furthermore, resistance to two unrelated viruses in this picture means that there is a tie-up between the synthetic chains which lead from a gene to the substances required by these two viruses, respectively. The viruses themselves need have no similarity with each other. These ideas have been explained more fully in a recent paper by E. H. Anderson (1).

While we thus lose the cross-resistance test for the classification of the *viruses*, we can still retain the virus-sensitivity tests for the classification of *bacteria*, as in Table I. Tests of this kind have been used for the classification of naturally occurring strains of many species of bacteria. On the whole, the "typing" of an unknown strain by its virus-sensitivity pattern seems to be about as satisfactory a method as any other, and it seems to agree with serological typing.

Here, however, we are more interested in the possibility of classifying, not arbitrarily selected wild strains, but the family of mutants of one strain. With how much assurance can we here recognize identical mutants by their sensitivity pattern? An important piece of evidence on this point comes from the work of Demerec and Fano (3). These authors find that the same sensitivity pattern may be reached either in two mutations or in one mutation. Is the end product here genetically alike in the two cases, or only phenotypically? If the first, we would have to assume the existence of coupled mutations; if the latter, we might hope to identify phenotype differences by further tests, either with other viruses, or by physiological studies. Until such studies have been carried much further we can not form an opinion on the merits of the sensitivity tests for purposes of classification.

TABLE II

RESISTANT COLONIES OBTAINED WHEN PLATING HIGH TITER STOCKS OF VIRUSES ON B/1 AND B/5

	T1	T2	T3	T4	T5	T6	T7
B/1	Inf	4	233	275	Inf	38	518
B/5	Inf	14	200	250	Inf	20	600

Table II gives the number of resistant colonies obtained when plating these two strains with any of the seven viruses. Both B/1 and B/5 give complete growth when plated with high titers of T1 or T5, since they are resistant to these viruses. With the other viruses they give a fairly characteristic number of mutants, and it will be seen that the number of mutants obtained from B/1 is in all cases quite similar to the corresponding number obtained from B/5. What we are here comparing is really a portion of the mutational pattern of these two strains, and the parallelism might be considered strong evidence for the similarity, if not identity, of the strains.

Again, however, the results obtained by Demerec and Fano (3) should make us cautious before accepting this similarity of the mutational pattern as evidence of genetic similarity. In very extensive tests these authors could show that the mutational pattern is remarkably similar in bacterial strains which differ by one or two mutational steps. Similar results were found by Luria (2) and by E. H. Anderson (unpublished). Since, therefore, the available evidence shows that mutational patterns may be similar in strains with known differences, we cannot accept the similarity of the mutational pattern as a criterion of genetic identity.

MATHEMATICAL APPENDIX

I. Population dynamics.—

We wish to prove in this Appendix some of the quantitative statements made in the text. This will help to clarify their meaning and the limits of their applicability. The results are probably not new, the proofs perhaps somewhat simpler than those given previously. All equations refer to average values and are therefore of practical value only for the very high mutation rates where the fluctuations mentioned in the text are not the dominant feature.

Notation:

N = number of normal type bacteria at time t.

M = number of mutant type bacteria at time t.

a = forward mutation rate, defined as the fraction of normal type bacteria mutating per time unit.

b = reverse mutation rate, defined as the fraction of mutant type bacteria reverting to normal type per time unit.

k_N = growth rate constant of normal type bacteria, defined as the fraction of normal type bacteria which divide per time unit.

k = growth rate constant of mutant type bacteria, defined as above.

$s = k_N - k_M$ selection rate of normal against mutant type.

From these definitions follow the basic equations:

$$dN/dt = (k_N - a)N + bM$$

$$dM/dt = (k_M - b)M + aN$$

Case a.	*Case b.*
Forward mutation balanced by selection, no reverse mutation $b = 0$	Forward mutation balanced by reverse mutation, no selection $s = 0$, $k_N = k_M = k$

For these special cases the equations (1) simplify, respectively, to:

$$dN/dt = (k_N - a)N \qquad dN/dt = (k - a)N + bM$$
$$dM/dt = k_M M + aN \qquad dM/dt = (k - b)M + aN$$

By changing to logarithmic derivatives and simple transformations these equations are easily transformed to:

$$d(M/N)/dt = +a - (s - a)M/N \qquad d[M/(M+N)]/dt = a - (a+b)M/(M+N)$$

The following integrals of these equations are adjusted to satisfy the initial condition $M = 0$ at $t = 0$:

$$\frac{M}{N} = \frac{a}{s-a}\left(1 - e^{-(s-a)t}\right) \qquad \frac{M}{M+N} = \frac{a}{a+b}\left(1 - e^{-(a+b)t}\right)$$

In both cases the equilibrium values given in the text are found by putting t equal to infinity.

$$\left(\frac{M}{N}\right)_{equ.} = \frac{a}{s-a} \qquad \left(\frac{M}{M+N}\right)_{equ.} = \frac{a}{a+b}$$

II. Probability of extinction of a type which mutates with a frequency "a" per generation, without reverse mutation.—

Let us assume that we start a culture with one individual of the normal type. The normal type may then become extinct by mutation of this individual before it divides; or, it may become extinct after the first division, if both offspring mutate before they in turn divide; or, if one of them mutates and the other divides and both its offspring later mutate, and so on.

We may ask what the total probability is that the clone generated by one bacterium will eventually contain none of the original type. It is obvious that the original type is certain to die out if the mutation rate is greater than .5 per generation, because then the net growth rate of the type is negative; that is, on the average the type loses more individuals by mutation than it gains by reproduction in each generation. The probability of extinction is therefore 1 for a mutation rate of .5 per generation, or any greater mutation rate.

However, also for mutation rates smaller than .5 per generation the type may die out by chance during the early stages of reproduction. The total chance may be calculated by a very simple argument. We wish to calculate the chance of extinction of the normal type in a clone which starts with one bacterium of the normal type, just after the birth of this bacterium. This chance will be a function of the mutation rate "*a*." Call the function $L(a)$. We calculate it by making use of the fact that any normal type offspring gives rise to its own sub-clone, and

that the chance of extinction within this sub-clone must be equal to the chance of extinction in the whole clone. This fact allows us to set up a simple equation for $L(a)$. Let us consider events up to the moment of the first division. Up to this time the chance of extinction is a, the chance of survival $1 - a$. In case of survival up to this point two normal type bacteria will be formed by division, and the chance of extinction for each of their sub-clones is again $L(a)$. The chance that both sub-clones will die out is, therefore, $L^2(a)$. The total chance that the whole clone dies out is, therefore,

$$L(a) = a + (1 - a)L^2(a)$$

This quadratic has two solutions, namely,

$L(a) = 1$ which is valid for a greater than .5

and $L(a) = a/(1 - a)$ which is valid for a smaller than .5

The complementary chance, that of survival of the type, is

$$S(a) = 1 - L(a) = (1 - 2a)/(1 - a)$$

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GENETICS OF BACTERIUM-BACTERIAL VIRUS RELATIONSHIP[1]

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VIRUS-RESISTANT BACTERIAL MUTANTS

The problem of the modifications in host-virus relationship between bacteria and bacterial viruses is pertinent to the topic of this conference in many ways. First, it involves a problem of bacterial variation, namely, the study of hereditary changes in bacterial sensitivity to viruses. Second, it involves the study of a group of virus mutations, and of their relation to mutations of the host cells. Third, it leads us to a consideration of the mechanism of virus reproduction inside the host cell, which we may consider as more or less directly bearing on the mechanism of gene reproduction, particularly in view of the possible relationship between viruses and genes, which has been the subject both of older and more recent speculation.

From bacteria sensitive to a bacterial virus one can isolate virus-resistant variants, which develop after the bulk of a sensitive culture has been destroyed by the lytic action of the virus. In most cases, these resistant bacteria do not adsorb the virus; they grow in a perfectly normal way in its presence. According to d'Hérelle ('26) these resistant bacteria would stem from cells which, upon infection by virus, had acquired an hereditary immunity to it. Several other authors (Gratia, '21, Bail, '23, Burnet, '29) have maintained that virus resistance originates by a process of bacterial mutation independent of the action of the virus.[2] In some particular cases, in which resistance appeared frequently and was associated with changes in other properties, Burnet ('29) actually succeeded in isolating resistant variants in the absence of any virus. For the most common cases, in which the resistant individuals are a small minority, detectable only after lysis of the bulk of the bacterial population, a decision on their mode of origin can be reached by an indirect method (Luria and Delbrück, '43).

This is based on the analysis of the distribution of variant bacteria in a limited series of cultures started from few normal individuals. Variants originated by mutation multiply, producing clones of mutant bacteria, and the distribution of the numbers of mutant individuals can be predicted with some approximation on the basis of an analysis of the frequency distribution of the clones of various sizes. The approximately calculated distribution can be compared with the experimental one, and from the latter a fair estimate of the mutation rate is obtained by simple formulas.

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[1] Aided in part by a grant from the Research Fund of the Graduate School, Indiana University.

[2] An exception may be represented by some poorly understood cases of "lysogenic" resistance (virus carried and secreted by resistant bacteria) in which the resistance would appear to be the result of the established symbiosis (Burnet and Lush, '36).

This method was applied to the study of the origin of virus-resistant variants of *Escherichia coli* strain B, showing their mutational origin independent of the action of the virus. The latter simply acts as a selective agent, destroying the sensitive cells and revealing the presence of the resistant mutants. Very low mutation rates can be detected and measured with relative accuracy; for the case of mutations to resistance to virus *a*, the rate is of the order of 10^{-8} mutations per cell per generation.

A bacterium may be sensitive to several virus strains. Resistance to one or more of these may arise without affecting the sensitivity to others (Bail, '23, Burnet, '29). In some cases, resistance to two or more viruses may regularly be associated. A systematic study of the acquisition of resistance by *Escherichia coli* B to seven viruses (Demerec and Fano, '45) proved that resistance to a group of viruses may arise as a result of either one or more mutations. Particularly important seems the conclusion that the rate of a certain mutation is the same even when the mutation occurs in strains genotypically different because of previous mutations to resistance to other viruses. This suggests that different mutations may take place in different centers, as in the case of mutations in different genes of higher organisms.

It is interesting that the mutations to virus resistance are often associated with changes in other properties, although many of the mutants appear to differ from the parent strain only in virus sensitivity. Colony type (rough or smooth, Arkwright, '24, Hadley, '27), antigenic properties (Burnet, '29), growth rate (Delbrück and Luria, '42) may be changed. Recently, Anderson ('44) found that several mutants from *Escherichia coli* B require an unidentified growth factor, present in meat or yeast extracts and not needed by the parent strain.

These associated changes are evidently the result of the same mutation causing virus resistance. Some of them may actually be different manifestations of the same structural change in the bacterial surface. On the basis of studies on the relation between antigenic properties and virus sensitivity, Burnet ('30) suggested that the surface receptors for viruses are carried by the same groups carrying the heat-stable agglutinogens of the bacteria. This conclusion was supported by the study of a "virus-inactivating agent," extracted from virus-sensitive bacteria, which proved to be one of the polysaccharide antigens of the bacterial cell (Gouch and Burnet, '34). According to Burnet's idea, the common carrier of antigenic properties and virus sensitivity has an "antigenic pattern," more stable, and a "virus receptor pattern," which can undergo smaller changes, some of them not affecting the antigenic specificity.

To explain simultaneous changes in virus sensitivity and growth-factor requirements, Anderson ('44) suggested that the normal process of synthesis of the growth factor has a common link with the process of synthesis of the virus receptor. Simultaneous acquisition of resistance to several viruses, according to Anderson, may depend on mutations affecting some common step in the synthesis of the relative receptors.

VIRUS VARIANTS ACTIVE ON RESISTANT BACTERIA

Up to this point of our story, the bacterial viruses have played the role of selective agents whose action reveals the presence of the resistant bacterial mutants. The viruses, however, can themselves undergo hereditary changes in their capacity of attacking bacteria. From some of the viruses we succeeded in isolating new strains of viruses, very similar to the normal strains and still active on the original host, but capable of attacking also bacteria resistant to the normal viruses (Luria, '45). Thus, one witnesses the appearance of a virus variant endowed with a new property, namely, "virulence" or activity toward one or several mutant bacterial strains resistant to the parent virus. Similar virus variants had been noticed by Sertic ('29), and evidence of their occurrence can be found in some data reported by Burnet (1930, see Table II, p. 655).

From the viruses a, γ, T3, T6, and T7, active on the strain *Escherichia coli* B, we have isolated in pure form a series of virus variants, called a', γ', T3′, T6′, and T7′; some of these, particularly viruses a' and γ', have been studied in detail.[3]

Virus a' and virus γ' are indistinguishable from their parent viruses in every respect but their activity on resistant bacteria. Serological tests by cross-neutralization with antisera against a normal virus and its variant fail to detect any difference. The x-ray sensitivity, related to the particle size, is unchanged; this is also true for viruses T6 and T6′. Viruses a and a', and viruses γ and γ', behave identically in their action and growth on the common host B. Adsorption rate, interval between infection and lysis, and average yield of new virus per bacterium are identical for each pair of viruses.

Virus γ' seems to be active on all B/γ strains. Virus a' attacks a group of bacterial strains, B/a_2, resistant to virus a; it does not attack several other a-resistant strains B/a_1. It is interesting to notice that the bacterial strains B/a_2, sensitive to virus a', belong to the group which was found to require a growth factor not needed by strain B (Anderson, '44). The growth-factor requirement produced by mutation is therefore correlated with resistance to virus a, but not with resistance to virus a'.

The new hosts adsorb the variant viruses at a smaller rate than do the normal bacteria B. Another difference is the yield of virus per bacterium at lysis, which is significantly smaller for the new hosts.

In some other cases the virus change also affects the activity on the original host. Virus T6′, variant from T6, is poorly adsorbed, not only by its new host B/6, but also by the normal host B, which adsorbs virus T6 very rapidly.

By submitting bacteria to lysis by the variant viruses, we isolated a series of

[3] The notation for viruses and for virus-resistant bacteria used in this paper is a combination of those used by Luria ('45) and by Demerec and Fano ('45). For example, a bacterial mutant isolated in presence of virus a, and resistant to this virus, is called B/a; a mutant from B/a, isolated in presence of virus T6, will be called B/a/6. Sub-indexes indicate different bacterial strains isolated in presence of the same virus: B/a_1, B/a_2. Viruses a and γ are called viruses T1 and T2 in Demerec and Fano's notation. It is to be hoped that a generally satisfactory notation will be agreed upon in the near future.

new mutant bacterial strains resistant to them. For viruses a' and γ', the results can be summarized as follows:

A bacterial strain resistant to a variant virus is also resistant to the parent virus. Bacterial strains resistant to a normal virus, as we mentioned above, may or may not be resistant to its variants. Mutations to resistance to unrelated viruses, normal or variants, are generally independent. Exceptions are found: for instance, a strain resistant to viruses a and a', after being lysed by virus γ', showed the presence of a mutant resistant to viruses γ and γ' but sensitive to a and a'. A mutation here produced resistance to a group of viruses and return to sensitivity to another group.

It is useful to compare the range of activity of a variant virus not only with that of its parent virus, but also with that of other viruses. Similarity of host range may conceivably be an indication of genetic relatedness, unless it is proved that it can be acquired by convergent changes in the viruses. The following scheme illustrates the situation concerning viruses a, a', and T5, the latter quite probably unrelated to the others (different particle size, no serological cross-reaction). S = sensitive; R = resistant.

TABLE I

Bacterial strain	a	a'	T5	Growth-factor requirement
B	S	S	S	−
B/a_2	R	S	S	+
B/a_1	R	R	R	−
B/a_2/a'	R	R	R	+

The scheme shows that virus a' and virus T5 have the same host range, in spite of their unrelatedness. The change $a \rightarrow a'$ makes the virus similar to T5 in its activity range. The scheme also shows that two bacterial strains which have acquired the same range of sensitivity by one or by two mutational steps are not identical, phenotypically as well as genotypically. The study of growth-factor requirement reveals a difference which virus-sensitivity tests alone could not detect.

VIRUS MUTATIONS AND THE MECHANISM OF VIRUS MULTIPLICATION

The virus variants are detected by putting large amounts of a normal virus in presence of resistant bacteria on a solid medium: some colonies of virus (lytic plaques) develop, containing variant virus. As in the case of the origin of the resistant bacteria, we may consider alternative possibilities. On the one hand, the virus variants may originate by mutation during the growth of the normal virus on sensitive bacteria. On the other hand, they might represent the result of an

adaptation on the part of some particles of normal virus which, when in presence of resistant bacteria, succeed in attacking them, thereby acquiring an hereditary change in activity (Sertic, '29).

If the variants arise by mutation in the course of the growth of normal virus on sensitive bacteria, they will continue to multiply like normal virus particles (at least for viruses α' and γ') producing clones of variant particles. As in the case of bacterial mutations (Luria and Delbrück, '43), this clonal grouping leads to the expectation of very large fluctuations in the numbers of variant particles found in a limited series of similar virus cultures, each originated from normal virus particles.

Since the virus grows in successive "bursts" from infected bacteria, we may expect either clones of at least the size of a full burst, or smaller clones if a bacterium infected by a normal virus particle can liberate a mixture of normal and variant viruses due to the occurrence of a virus mutation in its interior.

The grouping of the virus variants was tested, for viruses α' and γ', by counting the plaques of variant virus in series of similar cultures of the normal virus after plating each culture with bacteria resistant to the normal virus and sensitive to the variant. The results (Luria, '45) showed distributions characterized by large irregular fluctuations of the proportion of variants from culture to culture, distributions best explained by the hypothesis of a clonal grouping due to mutational origin of the variants. The resistant bacteria here act as selective agents permitting the multiplication of the mutant but not of the normal virus particles.

The experiments also showed a large proportion of cultures in which the total number of variant particles was well below the average yield of virus per infected bacterium (cultures with 1, 2, 3, . . . variant particles). These particles must derive from bacteria inside which a virus mutation occurred, and which liberated a mixture of normal and mutant viruses. In order to calculate the expected distribution of these mutants and the mutation rates, we need to know how the virus multiplies inside the bacterium: but this is as yet unknown. This consideration, however, suggests that, from the experimental distribution of the number of mutants, we may be able by the reverse analysis to get information on the mechanism of virus reproduction. The distribution will indeed be different according to the mode of reproduction. Figure 1 graphically shows some of the possible methods of virus multiplication and mutation.

In fig. 1A, the virus is supposed to multiply autocatalytically, as do bacterial cells in a culture. In such case, the distribution of the number of mutants (in clones smaller than a full burst) should be the one discussed by Luria and Delbrück ('43) for bacterial mutations.

In fig. 1B, the virus is supposed to multiply by successive replications of the infecting particle. The probability that a newly formed particle is a mutant does not affect the probability of the next particle formed in the same cell: each mutant arises by an independent event inside the cell, and the numbers of mutants

will show a Poisson distribution (always for values below the full yield of virus per bacterium).

Figure 1C assumes that each virus particle is produced by replication of the last particle formed. If one particle mutates, all the following ones produced in that bacterium will be mutants. A mutation may occur with equal probability at any time, since there is only one particle multiplying at any given moment; clones of any size between one and the full yield are equally probable, and the distribution of mutant particles will show an equal frequency of all these values.

Figure 1D assumes a combination of cases B and C, in which the multiplication proceeds by replication of either of the two last particles formed. The resulting distribution of mutants will evidently be a superposition of a Poisson distribution (mutations not followed by replication) and a constant frequency distribution (mutations followed by replication).

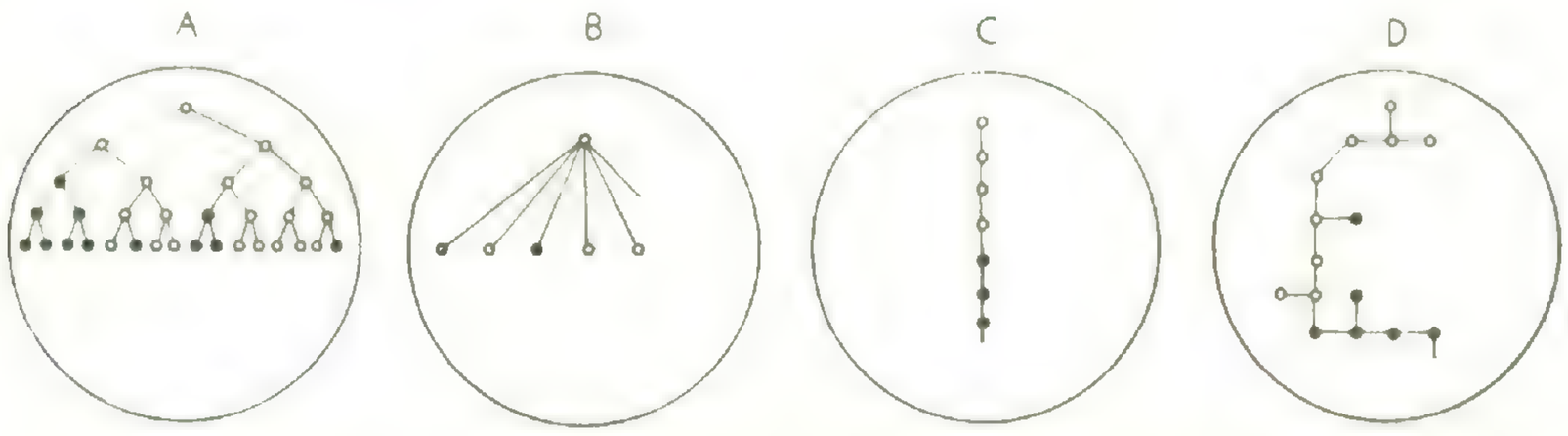

Fig. 1. Open circles = normal virus particles. Solid circles = mutant virus particles.

This type of analysis looks most promising, but unfortunately it has up to now met with a serious experimental drawback. Because of the poor adsorption mentioned above, the numbers of plaques produced by a mutant virus on the bacteria resistant to the parent virus is smaller than the true number of mutant particles by a factor generally around 0.3–0.8, but which can vary within the same experiment. The result is that one cannot obtain a sufficiently accurate experimental estimation of the distribution of the numbers of mutant virus particles. If this difficulty can be overcome, this type of analysis may prove the best way of approach to the problem of virus multiplication.

RELATION BETWEEN BACTERIAL AND VIRUS MUTATIONS

Some cases of abrupt changes (mutations) in bacterial viruses have been described before. The best-known case (Burnet and Lush, '36) concerns a virus carried in lysogenic form by a resistant staphylococcus. This virus gives a variant able to lyse the resistant bacterium from within, but still inactive on the resistant bacteria from without. A similar change was described by Gratia ('36) for a megatherium virus. In both these cases, the mutation affects the type of action of the virus on the bacterium rather than the ability to be adsorbed by and grow on resistant bacteria, as in our cases.

The fact that our virus mutants are adsorbed by bacterial mutants which do

not adsorb the parent virus focuses our attention upon the surface structures of viruses and bacteria. A change of the bacterial surface, making the cell resistant to a virus, can be compensated for by an independent change of the virus particle reestablishing the affinity of the surfaces. This fact suggests that the changes involved are small, probably consisting of simple stereochemical rearrangements. The low rate of adsorption of a mutant virus by bacteria resistant to the parent virus may be interpreted as due, either to less satisfactory "fitting" of the complementary surface structures, or to the fact that only one or few of several receptor groups on the virus surface have reacquired their affinity for the bacterial surface. It is not surprising that the antigenic properties of the viruses may not be affected by the mutations. For the virus particle, as for the bacterial receptors (Burnet, '30), we may conceive of an antigenic pattern more stable than the receptor pattern.

Given the small size and the composition of bacterial viruses (mainly nucleoprotein of the desoxyribose type), it is possible that the surface changes of the viruses actually represent the primary mutational changes. If so, the correlated changes of the bacterial surface would bear a similarity to the mutational changes in the virus. It is only a step to imagine that they are also closely related to the primary mutational changes in the bacterial cell.

The possibility that surface antigens of cells be structurally related to the genes which regulate their production has repeatedly been suggested (Irwin and Cole, '36, Haldane, '39). Sturtevant ('44) recently suggested the possibility that these genes may be reached and affected by antibodies against the antigens they determine. This possibility, offered in connection with experiments on *Neurospora* (Emerson, '44), is particularly enticing in the case of bacteria, where variation following antiserum treatment has repeatedly been reported. The surface structures involved in virus sensitivity, however, are possibly less direct products of the genes, since the same change in sensitivity is obtainable by different combinations of independent mutations (Demerec and Fano, '45; Anderson, '44). We have tried to increase the rate of bacterial mutations to virus resistance by treatment with antibacterial serum; fairly numerous attempts, however, have given as yet no indication of a positive effect.

A few words may be added on the bearing of these results on the problem of the origin of viruses. Because of their strict parasitism, it has often been supposed that they originate (possibly as "free genes" or "plasmagenes") from cells similar to those in which they can reproduce. We saw that host-virus relationship can be maintained by complementary, mutually compensating mutations. If the host mutations have evolutionary significance, as we have no reason to doubt, a virus parasitic to a certain type of cell may have derived, by a series of mutations, from a virus parasitic to a remote ancestor of its present host. Even if viruses originally derive from host cells, the genetic relationship between a virus and its present host may be very remote. The above consideration probably holds true for viruses other than bacterial viruses, since selection of virus mutants in the course of

adaptation to new hosts is probably of very general occurrence (see Findlay, '39, Burnet and Bull, '43).

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GENETICS AS A TOOL FOR STUDYING GENE STRUCTURE

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By making use of current ideas about the structure of genes it is possible to develop a formal scheme which will account for the continuity of genic specificity in gene reproduction, and for the initiation of gene-controlled reactions in the cytoplasm. Such a formulation gives a pattern into which many diverse genetic observations can be fitted and suggests definite lines of experimental approach which were not otherwise apparent.

Two characteristics of genes are important to the development of this scheme. On the one hand, genes differ greatly in their specificities as can be seen from the diverse gene-controlled reactions reported by Tatum and Beadle ('45) earlier in this Conference. On the other hand, genes are extraordinarily alike in all other respects, including their ultimate chemical constitution as understood at this time. This characterization of genes reminds one of similar properties of antibodies which resemble one another closely except in their specific relationships to their homologous antigens.

That antibody specificity resides in the unique surface configuration of a particular antibody molecule is strongly suggested by studies on the antigenic relationships of simple chemical substances (Landsteiner, '36; Marrack, '38). Pauling, Campbell and Pressman ('43) have pointed out how the specific surface of an antigen can serve as a template upon which the antibody surface is determined, the surfaces of the two molecules then being mutually complementary in shape and in the arrangement of reactive groups (i. e., oppositely charged groups, groups capable of forming hydrogen bonds, etc.).

Complementary, antigen-antibody like surfaces have been suggested for other biological systems. One of these is the relationship between enzymes and their substrates, such as the relations between specific glucosidases and chemically modified glucosides recently reviewed by Pigman ('44). From the antigen-antibody like reactions between the surface and underlying substances in certain invertebrate eggs, Tyler ('40) has suggested that such complementary structures may be an important feature in the architecture of all cells. On the basis of physical-chemical considerations, Pauling and Delbrück ('40) postulated that most biological syntheses involved the building of complementary surfaces, pointing out that complementary surfaces can be identical under certain circumstances.

While there is no direct evidence indicating that genic specificity resides in the unique surface configuration of the gene, there are several reasons for making that inference. In the first place, the specific active surfaces of enzymes (Tatum and Beadle, '45) and naturally occurring antigens (references in Emerson, '44) are themselves gene controlled. The simplest interpretation would be that surfaces of

the enzymes and antigens are derived from the surfaces of the genes involved. In the second place, genic specificity is transmitted through molecules of different chemical make-up. For example, the nucleoproteins in the chromosomes of the fish change during the ripening of the sperm from nucleo-histones to nucleo-protamines with no break in the continuity of genic specificity. This situation seems to me to be readily understood on the basis of surface configurations, especially when we recall that polysaccharide antigens determine the surfaces of gamma-globulin antibodies.

There are two general routes by which the surface structure of a gene could be transmitted in gene reproduction, and from the gene to the enzyme, or antigen. The gene could reproduce by forming a complementary, antibody-like template upon which the surface of the new gene is synthesized, or the specific surface could be copied directly if the gene has a structure like that proposed by Delbrück ('41; cf. Gulick, '44). The primary gene product in the cytoplasm could obtain its specific surface in either of these ways.

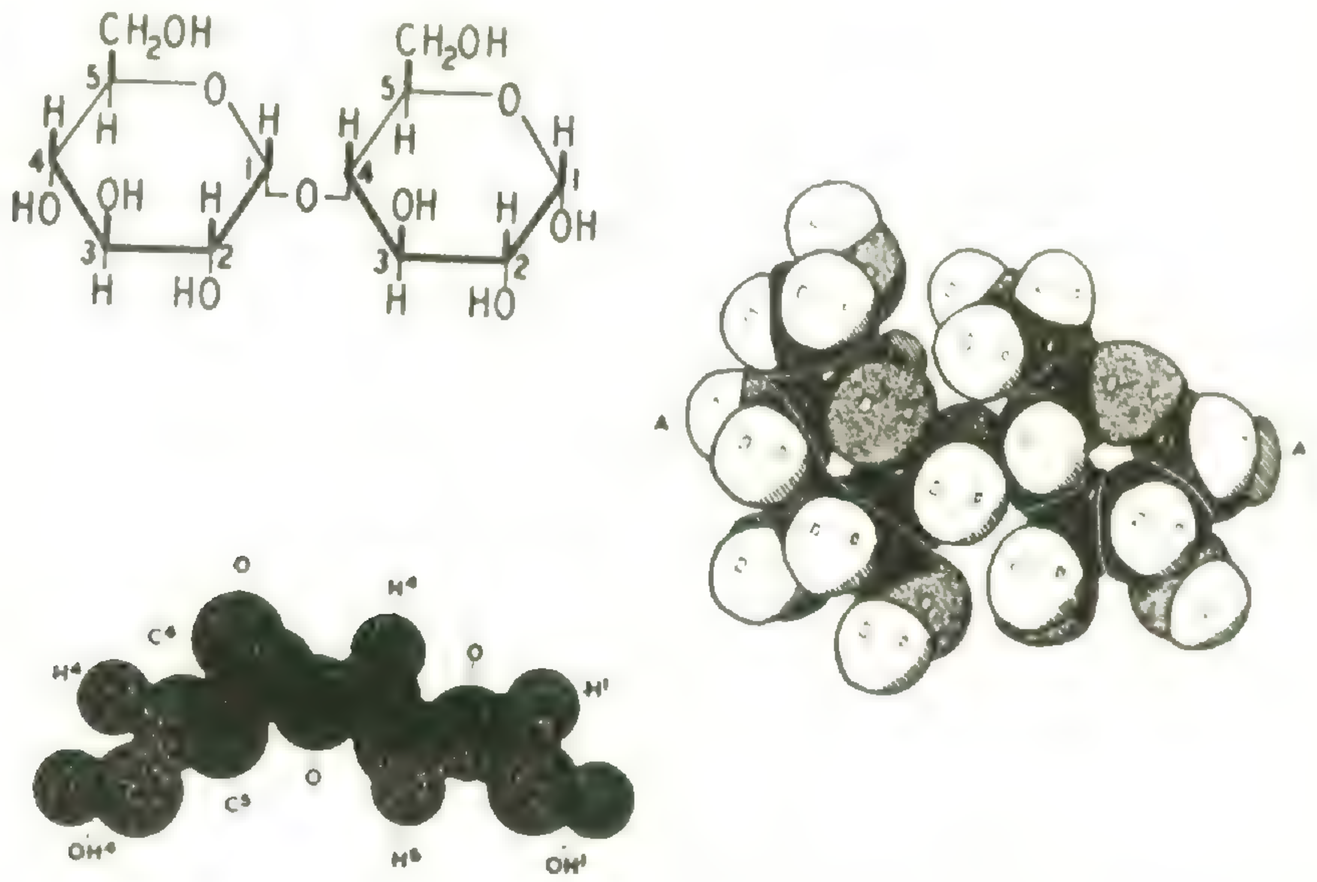

Fig. 1. Maltose molecule; upper left, structural formula; center right, surface view of model in same orientation as formula (black, carbon; stippled, oxygen; others, hydrogen); lower left, section through model, along line A—A.

These routes by which specific surfaces can be transmitted are shown schematically in figs. 1, 2, and 3, in which the gene determining the active surface of the enzyme maltase is used as an example. In the first figure a molecule of maltose is represented in three ways, by the structural formula, by a surface view of a model, and by a section through the model.

From what is known about the relationships between certain groups on the maltose molecule and the specific enzymes, it is possible to guess the surface of the sugar molecule which may be associated with the enzyme surface. Of special importance are the alpha position of the glucoside linkage (differentiating maltose from cellobiose) and the position of the hydroxyl group on carbon atom 4 of the

glucoside residue (which distinguishes glucosides from galactosides). Judging by the specificities of beta-glucosides (Pigman, '44), relatively minor substitutions on carbon atoms 2 and 3 of the glucoside radical would destroy the specificity, whereas minor substitutions on carbon atom 6 of this residue, or fairly substantial substitutions on the other glucose residue, should only lessen the specificity. These considerations make it seem probable that the enzyme attaches either to the upper surface (as illustrated in fig. 1), or to the lower surface. The upper surface has been chosen for purposes of illustration.

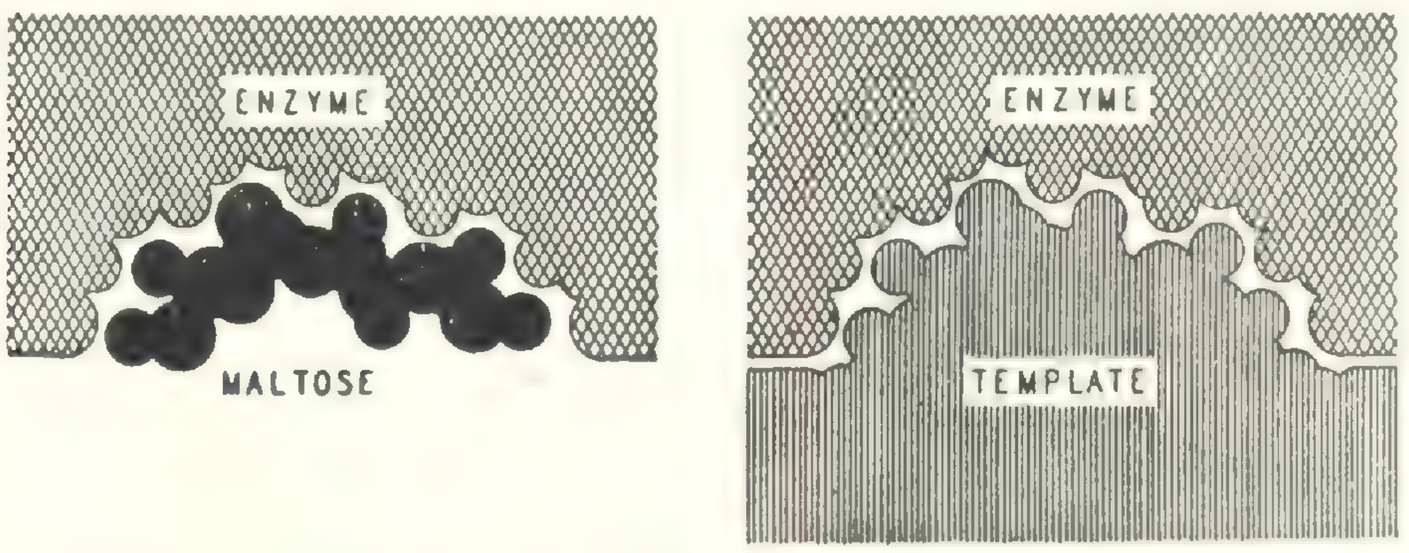

Fig. 2. Complementary surfaces: left, section through maltase (ENZYME) with associated maltose molecule; right, maltase and complementary template.

The diagram at the left in fig. 2 represents a section through a molecule of the enzyme maltase which has a molecule of maltose associated with its specific active surface. The surface drawn for the enzyme is not intended to be exact in any way, but is intended to represent a complementary relationship to the surface of maltose in the same sense as antigen and antibody molecules are complementary. At the right in this figure is a section through the same enzyme and a complementary template upon which the surface configuration of the enzyme might be determined. As drawn, the surface of the template is identical to the corresponding surface of maltose. They are not identical, one being a carbohydrate, the other presumably a nucleoprotein, but they must be similar to the extent that both are complementary to the surface of the enzyme.

Figure 3 is a scheme illustrating the possible routes of gene reproduction and gene action. The specific surface configuration of the gene (G) may be reproduced directly as shown by the dotted line, or indirectly through the intervention of a complementary template (T^G). If both gene and template are part of the genic material it is purely a matter of convenience which is called gene and which template. If the enzyme (E) has a surface configuration identical to that of the gene (upper half of figure) it may obtain this surface through a complementary template (T^E) or directly (dotted line), depending upon the structure and mode of synthesis of the molecules involved. The lower part of the figure shows how the enzyme might have a surface complementary to that of the gene, in which case there could be no surface in the genic material identical to that of the enzyme

unless the gene reproduces by means of a template.

Except for intermediate steps in gene action (which must duplicate the steps illustrated), this diagram exhausts the possible ways by which specific surfaces can be transmitted according to the postulates outlined above. It should be possible to distinguish between these alternative routes experimentally. Sturtevant ('44) and I (Emerson, '44) have pointed out how antibodies to natural antigens could cause mutations, provided the gene and antigen have similar surfaces, and we have interpreted certain examples on that basis. The argument used is briefly this:

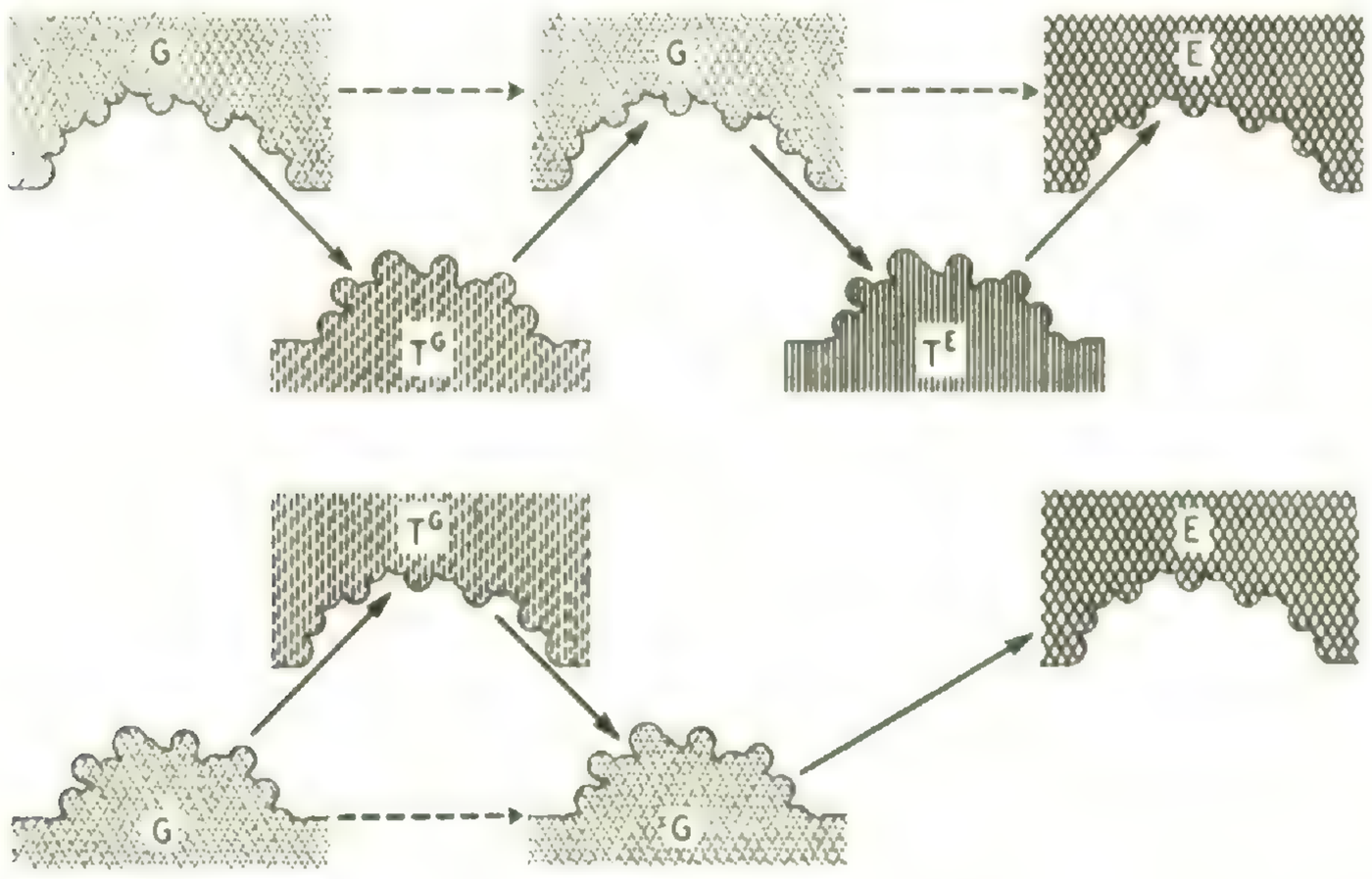

Fig. 3. Possible routes by which specific surfaces can be transmitted from gene (G) to gene and from gene to enzyme (E). T^G is a complementary gene template, T^E, an enzyme template.

that since the specificity of an antigen is genetically determined, gene and antigen may have identical surfaces, in which case antibodies developed against that antigen could combine with the corresponding face of the gene. The presence of the antibody molecule on the surface of the gene would so change that surface that the gene could no longer make an exact copy of itself, but would either fail to reproduce, or would produce a new gene with an altered surface. Either of these results would be recognizable as a mutation since the descendants of this cell could no longer elaborate the antigen in question. Experimental evidence is still inadequate on this point as there is no one case in which antibodies to a particular antigen have been shown to induce mutations in the gene responsible for the production of that antigen. If such evidence is forthcoming, it would indicate that the surface of the antigen is duplicated in either the gene itself or in the gene template.

If this scheme for the induction of mutations by antibodies should be correct, it should be possible to accomplish the same result more simply. For example, it is known that maltase activity can be inhibited by the presence of an excess of glucose. The glucose molecule fits into part of the active enzyme surface, and

when present in excess there is usually a molecule of glucose in the way of the maltose molecules which generally fit into this surface. By using very high concentrations of glucose it should be possible to have one of its molecules associated with the corresponding surface of the gene at the time of gene reproduction, resulting in mutation in the same way as when antibodies are present. We have made a few attempts along these lines by treating *Neurospora* with high concentrations of different sugars, analogues known to give substrate inhibition with certain enzymes, etc. Except for one mutation to be discussed later, the method has not proved too satisfactory, resulting usually in a great deal of sterility.

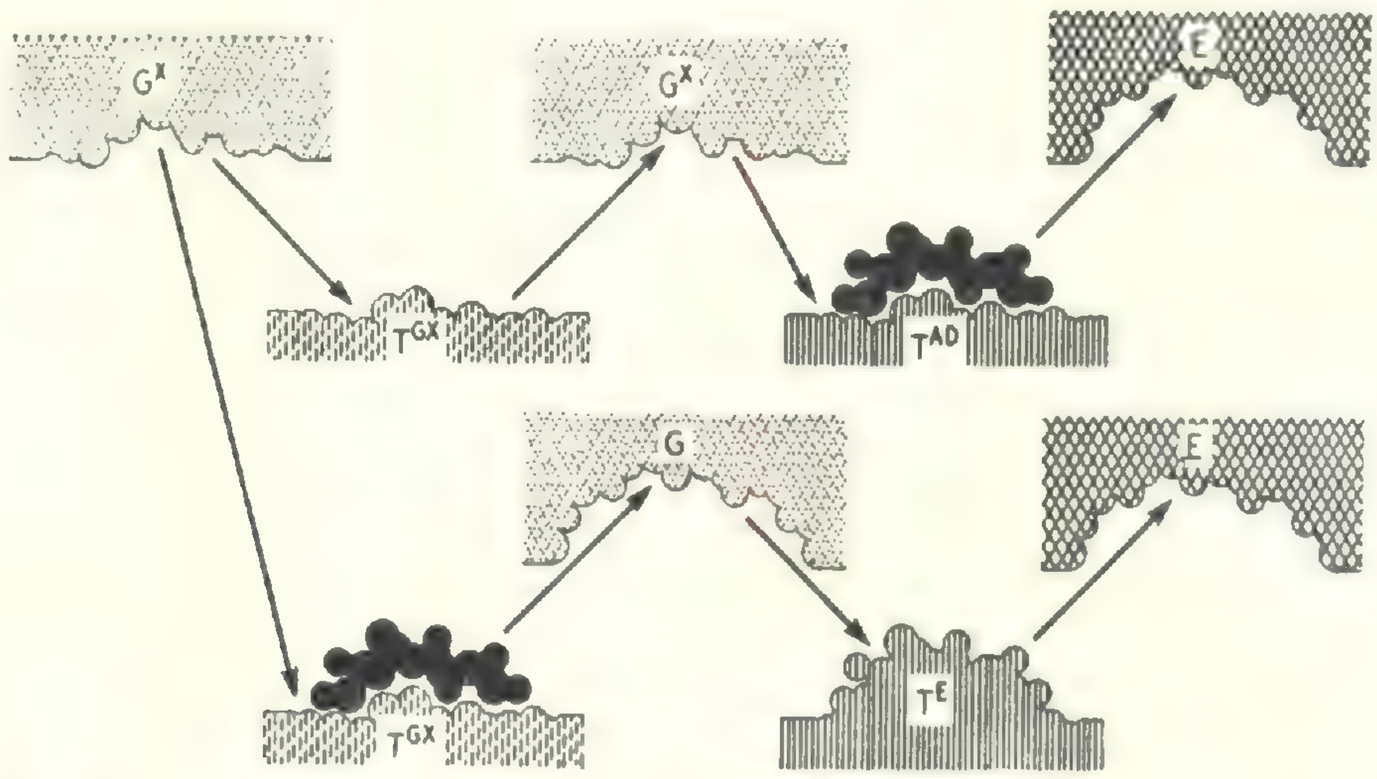

Fig. 4. Formation of adaptive enzymes: T^{AD}, adaptive template (maltose shown in in black); G^X, gene; T^{GX}, gene template; E, enzyme (maltase); G, mutated gene; T^E, enzyme (maltase) template. Further explanation in text.

The scheme just outlined can be extended to account for certain adaptive phenomena. The upper half of fig. 4 illustrates "cytoplasmic" or non-inherited adaptation. G^X represents a different gene from the one previously illustrated, and is shown as reproducing itself by means of a template (T^{GX}). The role of this gene is to produce a nucleoprotein template (T^{AD}) in the cytoplasm. In the presence of maltose, this template attaches to a molecule of maltose by its reverse side, and the surface of the template is altered in much the same way as the surface of an antigen is altered by the presence of a haptenic group. The enzyme that is synthesized on this template-plus-maltose will have a surface configuration complementary to the surface of maltose, which gives it the surface structure of the enzyme maltase (E). In this instance, maltase is an adaptive enzyme which is produced only in the presence of maltose. This is the way I had pictured the production of the killer substance in *Paramecium* as reported by Sonneborn ('43a, '43b), but from the additional evidence he has now presented (Sonneborn, '45) it is apparent that the scheme I have outlined will not account for all of his observations. Lindegren, Spiegelman and Lindegren ('44) have shown that genes are involved in producing a background for adaptive melibiose fermentation by yeast, giving a situation that fits the scheme outlined here, but to account for the addi-

tional observations just reported by Spiegelman ('45) it would be necessary to have the adaptive template (cytoplasmic factor) self-reproducing, as he has suggested.

The lower half of the same diagram (fig. 4) shows what must happen to the gene responsible for the adaptive structure under proper circumstances. If the organism is cultured in very high concentrations of maltose, enough molecules of the sugar should get into the nucleus so that some could combine with the gene-template (T^{GX}), and the gene then constructed on this template should produce maltase ever after, even in the absence of maltose.

Two examples that may represent mutations of this sort have recently turned up in our laboratory. By culturing *Neurospora* in the presence of sulfanilamide, Cushing (unpublished) was able to adapt a strain to the extent that conidia would germinate on higher concentrations of the sulfanilamide than the non-adapted strain, and, at a given concentration, mycelial growth was more rapid in the adapted strain. The adaptive characteristics were only partially maintained after a single subculture in the absence of the drug, and completely lost on outcrossing, indicating that the adaptation was not genetic. After growing the adapted strain on still higher concentrations of sulfanilamide, a heterocaryon was obtained which contained some normal nuclei and some mutated nuclei. Isolated mutant strains were extremely tolerant to sulfanilamide and in some respects made better growth in the presence of the drug than in its absence.

The second example is the one mutation obtained following treatment of *Neurospora* with a molecular solution of lactose. Lactase is an adaptive enzyme in *Neurospora* in that it is not produced in the absence of the specific substrate. The mutant differed from wild type in that it grew poorly on all carbon sources, but, in contrast to wild type, it grew just as well on lactose as on glucose, suggesting that lactase might be produced irrespective of specific substrate. Tests are now under way to determine if this is the case. The experiments to date do not distinguish between induced and spontaneous mutations.

While there is little available experimental material bearing on the mechanisms outlined, it may be useful to have them presented at this time. The scheme has many postulated steps, but each is fairly reasonable in the light of our present knowledge, and it does give a rather unified picture. There is the further point that the scheme should be amenable to experimental attack, and if the experiments are successful we have a way of learning something about genes from a different approach by making use of the methods of enzyme chemistry and immunochemistry.

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DISCUSSION

Following Reading of Lindegren's and Tatum and Beadle's Papers

Dr. Demerec: Yesterday I attended a seminar at the University of Missouri where Dr. Briggs from the University of California discussed the disease resistance of plants. He described four genes which determine resistance to smut in wheat and three out of those four are located in one chromosome. Now there are several instances where genes of like action are located in the same chromosome, and we have a number of cases where genes of very similar action are located very close together. I wonder if Dr. Beadle has any indication of instances where genes closely similar are located close together.

Dr. Beadle: There are a number of cases where they seem to be associated in ways that you might not expect. For example, there are two different albino strains that appear to be genetically different, yet they are so close together they practically never cross over. There is one chromosome, you may remember, concerned with three pyrimidineless mutants and I would hesitate to say whether they are chance distributions or not. You cannot take the chromosome map as a random sample. By our procedure we tend to pick up a non-random assortment of linkages.

Question: In some cases several genes may be involved in one very simple step of the over-all synthesis. That seems to indicate that those genes work together in the manufacture of the enzyme for that step. Is there any evidence that some other substance may be supplied which would enter into the formation of the enzyme?

Dr. Tatum: We have no evidence on that question. In the cases illustrated in which there are four mutations concerning the synthesis of arginine there must be four different steps involved.

Dr. Muller: Where you have two or more different mutations affecting the same synthesis and the substances are diffusible can you always get growth if you give the product of one of them to the other?

Dr. Beadle: Yes, if the substances are stable.

Dr. Stadler: Once you find the essential substance that is lacking how do you keep the culture growing?

Dr. Tatum: On a minimum amount; the cultures are given enough to grow, but no excess.

Dr. Cori: These intermediate products accumulate. To have them accumulate you stop formation of the final product. Why should the reaction continue in the intermediary stages if the final product is supplied?

Dr. Beadle: You don't have intermediates formed if you give a sufficient

amount of the final product, just enough to tease it a little. If you give plenty of adenine no pigment whatever is found, but if you give it just the right minimal amount, a large amount of purple pigment is formed.

Dr. Goddard: Dr. Tatum, how long does it take to bring about an adaptation that you can detect, a matter of hours, days, or longer?

Dr. Tatum: Ordinarily, it is a matter of days, not hours.

Dr. Goddard: Is there any relation between the amount of mycelium and adaptation time?

Dr. Tatum: I think there is; probably larger amounts of mycelium adapt more rapidly.

Dr. Goddard: I think there is a conceivable biochemical change important in adaptation and related to the volume of the tissue versus volume of the liquid. If you started from A and went to Z and had no intermediates, it would take an extraordinarily long time for anything to accumulate; time measured in terms of hours and not minutes. If, in a mutant, you suddenly have blocked one stage it might take a good many hours to build up sufficient concentration so a new reaction can start. This type of adaptation could not involve changes in existing enzymes or genes, but a real change in components. As James Franck pointed out, concerning a reaction which starts within a minute or so after illumination occurs, if all the intermediates between CO_2 and carbohydrates had to be built up it would take eight hours. As a consequence, one knows the intermediates must be present.

Dr. Delbrück: Can you tell us something about reverse mutations: whether they occur and can be recognized?

Dr. Beadle: The main difficulty in answering your question is the experimental difficulty in telling the difference between contamination and reverse mutation unless you have a special set-up because, of course, you would expect to get a wild type in reversion and you could also expect contamination by wild type.

Dr. Delbrück: It seems to me if this mutant can revert it should have a well-defined reverse mutation rate and should be distinguishable from an erratic contamination.

Dr. Beadle: If the mutation rate is high enough.

Dr. Delbrück: For spontaneous reverse mutation one could use Roepke's method of growing the mutants in the presence of minimal amounts of the missing factor and then see whether you finally get rapid growth.

Dr. Beadle: We have not done that extensively.

Dr. Hollaender: Have you found any difference between spontaneous mutation rates and rates produced by different types of radiation?

Dr. Beadle: We have not studied spontaneous mutation rate because, so far, we have not concerned ourselves with problems concerning mutation rate in

general. For one thing, we figured you could do a better job; also, we thought that the amount of testing we would have to do to determine the spontaneous mutation rate was so great we hesitated to undertake it.

DR. TATUM: I have a remark in connection with Dr. Goddard's suggestion. There is one mutant strain which requires thiazole and makes pyrimidine. This does not adapt to the slightest extent, no matter how long it is left. On the other hand, the thiaminless mutant strain which requires the intact molecule and which makes both intermediates adapts extraordinarily well. That might support this idea. However, in another instance involving pantothenic acid adaptation did not occur. So there are differences.

DR. GREENSTEIN: I would like to point out the very remarkable parallel between these effects that Dr. Tatum and Dr. Beadle have obtained and changes in neoplastic tissues. If one compares functions of normal living cells with those of tumors one finds similar reactions, but one must also assume that newer functions are induced. I wonder if Dr. Tatum and Dr. Beadle considered the possibility that mutant effects may have induced the formation of new functions, perhaps unnecessary, but present nevertheless.

DR. TATUM: I think there are pertinent examples of the formation of new substances in the production and utilization of intermediates and the production of pigments. It is perfectly possible that those are merely instances which we picked up; perhaps a whole series of new reactions are instigated by means of blocked reactions.

DR. MULLER: Would it be possible to test blocking any of the enzymes by anti-bodies?

DR. TATUM: I think that is quite possible to do. Perhaps Dr. Emerson has some comments.

DR. EMERSON: It would be easier to block with substrate; antibodies usually are not directed against the active groups on the enzymes.

DR. EDGAR ANDERSON: I would like to ask Dr. Beadle if he has information on chiasma frequencies.

DR. BEADLE: No, I haven't any, and I think Dr. McClintock has not made actual counts, although I think she could give you an estimate. I suppose the average is perhaps between one and two, possibly around two, but that is just a very rough guess.

DR. STADLER: I notice in the general discussion by Dr. Tatum that when there are, say, four genes affecting a step or sequence of steps, it is apparently assumed that it must involve four or more enzymes. I wonder if the results contradict the assumption that production of a single enzyme could be affected by two, three, or four genes?

DR. TATUM: We feel quite certain that there are a number of instances in which a number of genes will affect a common enzyme.

* * *

FOLLOWING READING OF SPIEGELMAN'S PAPER

DR. GODDARD: It would be of some importance to get at the nature of the enzyme-substrate compound. Do you know the Michaelis constant? Is the amount of galactose tied up with galactozymase dependent upon the galactose concentration?

DR. SPIEGELMAN: In attempting to obtain rate-concentration curves we ran into several difficulties. At high concentrations there is an inhibition of adaptation, probably osmotic in nature. At low concentrations it is difficult to keep the concentrations constant due to the preadaptive aerobic utilization of the galactose.

DR. GODDARD: The low concentration experiments are critical. Perhaps one might place the culture in a collodion bag suspended in a large volume of the galactose solution.

DR. BEADLE: Considering the four positive spores which segregate from the first hybrid, have you been able to demonstrate any difference at all in the two types, or have you looked for such a difference?

DR. SPIEGELMAN: As a matter of fact, a great deal of effort was expended in trying to find such differences. None was detectable. I might mention in passing that we tried to see whether the rates of adaptations differed between a haploid carrying two mel+ genes and one carrying only one such gene. Here again no difference could be established.

DR. BEADLE: In this diagram you have presented of gene action is it possible to suppose that the gene is originally determining the specificity of "P" which in turn somehow acts as a pattern for "E"? An analogous situation might be the transformation of pepsinogen into pepsin.

DR. SPIEGELMAN: I see no objection against a supposition of this kind. It will be noted, however, that it grants even more than I ask, that even in the presence of the controlling gene there exists a step in the chain which is autocatalytic and completely free of primary gene action.

DR. MULLER: I should like to raise the question of the specificity of the synthesis of "E", especially with relation to the gene. How likely is it to depend on factors other than this gene and "P"?

DR. SPIEGELMAN: You mean besides the mel+ gene?

DR. MULLER: Yes, other than the gene whose absence deprives it of the adaptation possibility.

DR. STADLER: I think the point mentioned by Dr. Muller is the one with which we are all concerned. It seems to me that the crucial question which must be considered here is whether we must, as a consequence of these experiments, add to our array of self-reproducing units a fundamentally new one; the self-reproducing enzyme molecule. Or, can we by not too complex or artificial a system construct a picture according to which we might account for the experiments without postulating a new self-reproducing unit? I know that Dr. Spiegel-

man has considered this question and I should like to ask him what assumptions he thinks are necessary to make it possible to explain the experimental results on a genic basis.

DR. SPIEGELMAN: We may assume the existence of a gene "X", different from either of the two mel+ genes already mentioned and capable, under certain conditions, of performing the same function. We must, in addition, ascribe the following properties to the "X" gene: it can only perform its function if kept in continuous contact with the enzyme. Thus the "X" gene would require the presence of the mel+ gene in order to start functioning. Once they were separated the "X" gene would remain functional only so long as sufficient enzymes were present in the cytoplasm; i. e., so long as the substrate was available. Another mechanism one might devise, although slightly more complicated, is that the gene "X" is stabilized directly by the substrate rather than by the enzyme. These are, I feel sure, not the simplest desirable mechanisms involving primary gene control. I am reasonably certain, however, that no mechanisms depending on genes will be able to avoid assuming that the functional stability of the controlling gene is dependent on either the substrate or the enzyme.

DR. GREENSTEIN: This hypothesis would seem to involve a serious revision of previously conceived notions of the nature of the gene. I wonder whether your reaction could not be modified somewhat by saying "P" plus "S" is equal to "E". In that way the character of your enzyme would be a function of your substrate.

DR. SPIEGELMAN: I don't see offhand that this is essentially different from the scheme I presented. The enzyme content is a function of substrate from the point of view of its stabilizing effect, and it is this aspect that I wished to emphasize. Evidence for a more direct involvement such as would be implied by the suggested modification is not available.

DR. GREENSTEIN: Are you certain that glucozymase is more stable than the galactozymase system?

DR. SPIEGELMAN: Yes. We have direct evidence in the form of both *in vitro* and *in vivo* comparisons.

DR. SONNEBORN: May I suggest that this other gene "X" we have been discussing seems to be excluded by the experiments in which removal of melibiose leads to loss of adaptability?

DR. SPIEGELMAN: The assumption of irreversible loss of function on removal of substrate (and enzyme) would explain the data.

DR. STADLER: It seems obvious that any other gene one would postulate would have to have some very artificial characters. The very nature of these characteristics could, by their artificiality, carry us a long way toward the necessity of assuming self-reproducing enzyme molecules. We may perhaps simplify the question under discussion if we stress the comparative plausibility of any proposed genic mechanism with that of the self-reproducing hypothesis.

DR. SONNEBORN: There is one difficulty with the genic mechanism. The "X" gene can apparently react to substrate when it is in the presence of the

mel+ gene but it loses that capacity when both the mel+ gene and the substrate are removed.

DR. SPIEGELMAN: If one is bent on retaining the gene mechanism it is not too difficult to get around this dilemma. One could assume that there is a sort of "position effect" in terms of a diffusible substance made by the mel+ gene required by the gene "X" for functional activity in the absence of substrate. Even simpler, it seems to me, would be to explain it in terms of interaction between gene "X" and enzyme, which latter we know is mediated by the mel+ gene.

DR. DELBRÜCK: Omitting for the moment the question of how one can avoid the self-duplication of enzymes, I should like to discuss another aspect of the problem. One property you have assumed which perhaps might at first glance appear peculiar is that the transformation from "P" to "E" can be catalyzed by two different agents, gene and enzyme. The first thing one would suspect is that perhaps "G" and "E" are very similar, a suspicion you have probably entertained. I might add that aside from these experiments and your formulation it is a suspicion one might have in any case, in view of the close correlation between gene and enzyme presented this morning by Dr. Beadle and Dr. Tatum. We would picture the present instance then in the following terms: you have a gene "G" which is stable so long as it remains in the nucleus. It produces an enzyme "E", similar to itself. When the enzyme gets into the cytoplasm it becomes unstable, a condition which, as you pointed out in the beginning of your talk, may be a very general one. Now if this unstable enzyme is stabilized by substrate then it can, in the cytoplasm, replace the gene. This seems to me a very fruitful picture. I do not, however, wish to lead you away from the search for alternative explanations.

DR. SPIEGELMAN: I should like to point out one difficulty I have encountered in trying to push the analysis along the lines suggested by Dr. Delbrück. If the genes and the enzymes they produce are similar why is it that the former are stable, whereas the latter are not? In terms of modern biochemical concepts the instability of the enzyme in the cytoplasm is the easier of the two to understand. All the components in the cytoplasm are in a state of flux and any given one can maintain itself only by balancing the rate of its disappearance with an equal rate of resynthesis. Here the substrate performs two functions, stabilizing the enzyme molecules and providing energy for synthetic activity. We have more or less direct evidence for this view. The rate of disappearance of enzyme in the absence of its substrate is proportional to the over-all metabolic turnover of the cells. Such unstable enzymes can be stabilized by depressing metabolic activity with, e. g., anaerobiosis or with sodium azide which prevents nitrogen assimilation. One way of explaining the stability of the gene on the same basis would be to assume that it is outside the metabolic cycle and that it alone of all protoplasmic units does not undergo continual breakdown and resynthesis. This raises the obvious difficulty of being forced, in a sense, to remove genes from the effects of

reactions which they control and with which they must necessarily be in intimate physiological contact.

DR. CORI: One fundamental question here is what is meant by reproduction of enzymes. What ideas do you have on this with respect to the mechanism and the nature of the precursor?

DR. SPIEGELMAN: I cannot go further at present than to say that the enzyme influences its own production. The details of the mechanism must await the elucidation of the nature of the precursor. With respect to the latter it seems to me unlikely that we are dealing here with synthesis all the way from the amino acids. It appears more likely that the precursor is an indifferent protein.

DR. BEADLE: I don't quite see the reason for the anxiety to avoid the concept of self-duplication of enzymes. In this connection, I should like to bring up again the pepsinogen-pepsin transformation and its relation to this problem.

DR. MULLER: I should differ with Dr. Beadle in just one respect. I think there is evidence here of self-reproduction; something akin to the gene. However, we do not know how general this phenomenon is. The concept is sufficiently novel to require more proof before its generalization is accepted. With respect to the pepsin formation it must be noted that it requires a very specific precursor, pepsinogen.

DR. BEADLE: How do we know that we do not have a specific precursor here much like pepsinogen? Wouldn't we then have what appears to be self-duplication in terms of transformation from precursor?

DR. GREENSTEIN: Pepsinogen is an inactive form which can be activated by either pepsin or hydrochloric acid.

DR. BEADLE: Couldn't "P" be an inactive form of "E"?

DR. GREENSTEIN: Perhaps, but it is an inactive form whose ultimate activity is dependent upon the types of substrates available at any one time. If we regard the proteins as the precursors, then their final enzymatic activity would depend on the available substrates and the genes would provide a *modus operandi* to allow it to proceed in the direction permitted by the existent conditions.

DR. BEADLE: Perhaps I should put the question in a different way. Is it possible that in the self-duplication of genes in general there exists a common inactive form which can be transformed in many ways to make different genes?

DR. MULLER: All the evidence we have points to a very general "precursor" for genes. The most essential property of a gene is expressed in its capacity to produce an exact duplicate irrespective, within wide limits, of associated genes or environmental conditions.

DR. SPIEGELMAN: There are several aspects of the pepsinogen transformation as an analogy that I would like to mention. The formation of pepsin from pepsinogen is spontaneous if pepsin and hydrogen ions are available, i. e., no energy is required. This is not true for the enzymes we are considering. Removal of the source of energy supply leads to cessation of enzyme formation. As far as the genic aspects of the problem are concerned, it seems to me little is gained by

the analogy. Pepsinogen already contains inherently all the specificity of pepsin. Thus the analogy would say that what we are observing in the kinetics of adaptation is a simple autocatalytic activation of inactive galactozymase or melibiozymase. Presumably the main function of the gene is specificity determination of the enzyme it controls. We would thus leave unexplained how the inactive forms of the enzymes with their inherent specificities could be synthesized in the absence of their specific genes.

DR. LINDEGREN: Isn't it true that a direct application of this analogy would mean that there was always available a considerable amount of this specific precursor even in the unadapted cell? This does not seem to be a reasonable situation.

DR. SPIEGELMAN: In this connection we might mention that when one tries to induce two enzymes simultaneously a competitive interaction can be established. This could be interpreted as competition for a common substrate which goes into the formation of the two different enzymes and would argue against any great specificity of the precursor.

DR. TATUM: It could be a competition for a limited source of energy.

DR. SPIEGELMAN: That is certainly true.

DR. HERSHEY: And it might be noted that shifting the gene action to the reaction forming "P" does not change the nature of the question.

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FOLLOWING READING OF HOLLAENDER'S PAPER

DR. RAPER: I might amplify just a bit what Dr. Hollaender was saying about this Aspergillus work. To test organisms for itaconic acid is time-consuming. Dr. Beadle does not know how fortunate he is in tracing down certain vitamins. Among the large number of cultures tested were about fourteen we thought particularly interesting. One culture did not increase the yield of total acid, but gave itaconic acid of exceptionally high purity. The other thirteen cultures did give total yields higher than the average for all of the controls which were run along with them, and of this number there were one or two that gave increases of 15 to 20 per cent. That is not a spectacular increase, but it is significant.

At the same time we have been isolating strains from nature. About 330 or so of these have been tested, and we now have a dozen or more that are better than our original stock. This merely goes to show that we did not have the best organisms to start with.

Dr. Hollaender alluded to the fact that they came from Texas. The majority have come from soils obtained from Texas, Arizona, New Mexico, and whether it means anything or not, Calcutta, India, and Western Australia. The temperatures are fairly high and the climate rather dry, a fact that may be of interest to you.

I would like to ask Dr. Hollaender whether the penicillin production tests

were run in submerged culture.

DR. HOLLAENDER: Yes.

DR. RAPER: Assays showed maximum yields at three days?

DR. HOLLAENDER: Yes.

DR. RAPER: Maximum yields at three days are a little unusual in the type of equipment with which we work. I do not know whether it is Dr. Beadle's experience or not.

DR. BEADLE: We would agree with you that they would come later.

DR. RAPER: I would raise one slight question about some of the yields. On the second-, third-, and fourth-day assays you would not expect the tremendous jump and then the abrupt fall shown in some of these figures. They jump from 18 to 60 to 20 on the second, third and fourth days respectively. One would expect that the slopes of that curve would be more gradual as a general thing.

DR. HOLLAENDER: We have data on five-day cultures that show fairly high yields but not so high as the three-day cultures.

DR. MULLER: If we assume that a given product, such as penicillin, has some adaptive value to the organism, and if we could find out the conditions under which it would help the organism, then by increasing the intensity of those conditions it might be possible to establish some sort of automatic selection. For example, if the penicillin helps to protect the Penicillium against competing bacteria, the addition of bacteria to a lot of cultures might produce such a condition.

DR. GODDARD: That was proposed some time ago and has been tried out in our laboratory and other laboratories, but we do not know of an actual stimulation or increase in productivity.

FOLLOWING READING OF GREENSTEIN'S PAPER

DR. STEINBACH: It is a rather interesting point that, as you say, you get the interaction between the nucleic acid and the globular proteins. The general tendency is to think of the protein chromosome matrix as more or less fibrous.

DR. GREENSTEIN: Since fibrous protein may at the same time be rather rolled the difference between fibrous and globular proteins may not be critical.

DR. STEINBACH: Is this interaction of certain nature? For example, if you are building up a chromosome is the nucleic acid a part of a definite pattern, stuck on the sides, or what would it be?

DR. GREENSTEIN: It is fixed only at fixed moments. The chromosome is not a stationary object; one must assume that it is changing continuously and one could expect that the physical properties of each of the components would be changed at each moment.

DR. SPIEGELMAN: What groups fix? Where does the interaction take place between the protein and the nucleic acid?

DR. GREENSTEIN: It must take place between charged groups, and this can have a marked effect upon the shape of nucleic acid.

DR. STURTEVANT: How combinations occur is of interest to geneticists who study specificity at different levels. Now, frequently it has been customary to think of genetic specificity as due largely to the protein. I take it that the current tendency is perhaps to ascribe it to the nucleic acid component. I wonder if you would be willing to throw some light on that question.

DR. GREENSTEIN: Nucleic acid, although of relatively simple structure, incapable of such alterations as proteins are, nevertheless can attach itself to certain types of proteins and produce an over-all difference in specificity. I don't quite see how a nucleic acid by itself can act; it must produce a combined effect. I think we are both coming to the conclusion that nucleic acid perhaps exerts its effect by contributing to reacting systems.

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FOLLOWING READING OF SONNEBORN'S PAPER

DR. SPIEGELMAN: I should like to suggest, Dr. Sonneborn, that the possibility of self-duplication of kappa is not entirely ruled out. Your conclusion depends on the fact that without the K gene the kappa disappears, but if we should assume that the precursor is always limiting, the amount of kappa that could be formed at any given time by self-duplication would be small. The velocity going from precursor to kappa would then be gene-controlled and you would always need the gene there to maintain kappa.

DR. SONNEBORN: Would you suggest any method by which it could be detected?

DR. SPIEGELMAN: Well, unless you know the precursor you cannot test it; that is true.

FOLLOWING READING OF GOWEN'S PAPER

DR. DELBRÜCK: You cited one instance of a spontaneous mutation, rate 1 in 180, and you determined the presence of the mutation by looking at the colony?

DR. GOWEN: Taking one of these micro-colonies and then plating out 100,000 cell groups on the assumption that you would have equal rate between the mutant type and the original type.

DR. DELBRÜCK: But you determined the character, smooth or rough, after your bacteria had grown into a colony?

DR. GOWEN: That is right.

DR. DELBRÜCK: How do you know the mutation does not occur in the colony?

DR. GOWEN: I don't. I expect it does occur in the colony.

DR. DELBRÜCK: Perhaps I misunderstood the procedure: Take one bacterium, let it grow into very small colonies; then pick out and plate individuals from the small colonies, letting each individual grow into a colony, and now you determine

whether these latter colonies are rough or smooth?

DR. GOWEN: Yes.

DR. DELBRÜCK: If the mutation rate is anywhere near 1 in 180 then all the colonies must be sectorial colonies because in each colony you grow individuals up to many millions.

DR. GOWEN: Rough and mixed, and some smooth, we think.

DR. DELBRÜCK: A colony consisting of one million smooth bacteria if your mutation rate is 1 in 180?

DR. GOWEN: I said we think they are smooth. If your mutation was toward the end of the process of course you would not be able to distinguish it.

QUESTION: How many cell generations do you have?

DR. GOWEN: About thirty minutes is the generation time.

DR. SPIEGELMAN: The experience I had some time ago with S. *aertrycke* bears out Dr. Delbrück's contention: we had a rapidly varying strain where rough gives off rough and smooth; when the mutation to the rough was high we got all rough.

DR. GOWEN: You were sure you started with rough colonies?

DR. SPIEGELMAN: Yes.

DR. GOWEN: We have not found many of those. In fact, offhand, I do not think I know of any with that particular set of characters.

DR. MULLER: It seems to me that the method will work if you have enough colonies, if you allow for the number of generations, and assume symmetrical reproduction. Can't you work it that way if you do enough colonies?

DR. GOWEN: That is right. Don't think for a minute that I think this technique is as good as micro-dissection technique, but after you work with the microscope for a year you also turn to other check methods as well.

DR. DELBRÜCK: I don't see how the micro-dissection technique can be of any help in determining mutants only recognizable in the character of the colony.

DR. GOWEN: If you grow the colony large enough, of course, you will get rough and smooth out of it and, a point I did not bring out, this change occurs in one cell generation.

DR. STURTEVANT: Perhaps I missed something. I would like to know how you keep this mutant stock in the first place.

DR. GOWEN: It is only through very rapid transfer that you may keep it. If you leave it any length of time it becomes rough very promptly. It is possible to classify the original micropipette-selected bacterium as either of the smooth type or the rough type in genetic constitution, because the mutant form leading to rough colonies is stable. All the bacteria coming from the single micropipette-isolated bacterium which is itself rough will themselves give only rough colonies. On the other hand, the bacterium forming a smooth colony is unstable. Samples taken from a colony originating from such a smooth-type bacterium will show colonies which are smooth, smooth and rough sectored, and rough.

* * * * * *

FINAL DISCUSSION

DR. MULLER: Dr. Lindegren has asked me to give a more detailed picture of the hypothesis that I suggested yesterday, when I attempted to bring his and Dr. Sonneborn's findings under the same scheme. For this purpose let us consider the diagram here shown:

Here, at the beginning, you have Dr. Sonneborn's "Killer gene", a large "K", and this produces a certain gene product, π, which in turn is required for the production of the cytoplasmic substance kappa, κ. The latter, in our present scheme, is to be considered as corresponding with the "cytogene" of Lindegren. For reasons that will appear, it seems likely that the product π is closely related in its composition to the gene K that produced it, and also to the substance kappa, or "cytogene", that follows it. If so, π may be regarded as the precursor of kappa, and would represent the intermediate step in at least two successive modifications of K. It is not absolutely necessary for the scheme, however, to assume these relationships in composition of the three substances concerned.

Now, the presence of π would not by itself be sufficient, in either Lindegren's or Sonneborn's cases, for the production of kappa. But if some kappa is already present to begin with, the reaction $\pi \rightarrow \kappa$ is activated by this kappa, as indicated in the diagram by the curved arrow that arcs upwards and backwards from kappa and impinges on the arrow leading from π to κ. Thus more kappa, or cytogene, becomes formed. If π and κ are related in composition, this reaction might be compared (as has been done by others independently) to the effect of pepsin in transforming pepsinogen into more pepsin, although it is not yet known whether this type of self-activation is common for enzymes. However, on the assumption that K, π and κ are all related, this self-converting effect of kappa could be regarded as due to kappa having preserved in itself something of the nature of a gene. It would not have a gene's more generalized ability of converting non-specific materials of the medium into material like itself, but it would have a more limited ability of thus converting a specific precursor, one which had already gone a large part of the way in the shaping of the final material.

Now, if the gene K should be removed, the chain of reactions is broken, and no more kappa can be produced. This breakage has occurred in Sonneborn's material, by mutation of the gene K to k, but nothing analogous to this mutation has yet been found in Lindegren's material. Removal of kappa also, in Sonneborn's material, breaks the reaction chain, so as to stop further kappa production. But this is not always true of what Lindegren has called the cytogene in his material. In his case, even though this cytogene is in some lines necessary for the production

of more material of its own kind, in other lines, differing from the former in a single pair of genes, which we may here designate as E versus e, the final substance or cytogene can be produced even when there is none of it present to begin with. The gene E of yeast then, or a product of it, must here be able, like kappa itself, to activate the reaction $\pi \rightarrow \kappa$ so that this reaction will go on even in the absence of initial κ. If kappa (here the cytogene) were closely related to π in composition, it would not be surprising that the conversion could be effected by other means than by kappa itself, as is true also of the pepsinogen-pepsin conversion. (The alternative scheme, not relating the two sets of results, would tend to have the cytogene similar to E instead. In the *Paramecium* material, it is as though none of the lines discovered contain E, but only e (or the absence of any comparable gene).

Another difference in the two materials lies in the fact that, for persistence of the cytogene, but not, so far as known, for that of the kappa of *Paramecia,* a certain substrate, melibiose, is required. If the cytogene can be identified with the enzyme melibiose-zymase which enables us to recognize its presence, this requirement is quite understandable, as many enzymes deteriorate rapidly in the absence of their specific substrates. But this very requirement helps us to understand also the presence of E in the yeast material, since without E the cytogene would be permanently lost every time a line of cells happened to grow in a medium lacking this specific substrate. In *Paramecia,* where there seems to be no such specific external substances which are likely to be missing from the environment, there is no need for such a gene as E to have become incorporated into the reaction system.

Of course the scheme here outlined is merely one conceivable possibility for relating the results on the two different organisms. The test of it would lie in the proof or disproof of the conception that the cytogene, like kappa, and unlike the genes of the chromosomes, continuously depends for its production on certain specific material (π), derived from or due to a particular gene.

Annals
of the
Missouri Botanical Garden

Vol. 32 SEPTEMBER, 1945 No. 3

A REVISION OF THE GENUS SCHKUHRIA

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Schkuhria is a member of the tribe Helenieae of the family Compositae. The problem of the correct generic name for this group of plants was considered by a Special Committee for Phanerogamae and Pteridophyta appointed by the 6th International Botanical Congress, Amsterdam, 1935. The action of the committee relegates the *Schkuhria* Moench (1794) to synonymy under *Siegesbeckia*, rejects *Tetracarpum* Moench (1802), and validates *Schkuhria* Roth (1797). The type species is designated as S. *abrotanoides* Roth, Cat. Bot. **1**:116. 1797.[2]

Asa Gray was one of the first to make valuable contributions to the knowledge of the genus in his "Notes on Compositae"[3], and his later revision in "Contributions to North American Botany"[4]. The next extensive revision of the genus was P. A. Rydberg's treatment of the North American species for the North American Flora[5] in which eight species were recognized as belonging to *Tetracarpum*, and a new genus, *Cephalobembix*, was created for S. *multiflora*. The most recent work on the genus is A. L. Cabrera's excellent paper on the Argentine species[6]. All in all, the genus has never been monographed, and since the time of Gray has not been treated in its entirety.

The overlapping of morphological characters makes it difficult to separate several genera in the tribe Helenieae[7]. Nor has it been easy to separate *Schkuhria*

[1] In the fall of 1943 Mr. Norlan C. Henderson, a fellow student in the Henry Shaw School of Botany of Washington University, began a study of *Schkuhria*, but was unable to complete it because of conditions incident to the war. For his use the representation of this genus in the Missouri Botanical Garden Herbarium had been supplemented through loans from several of the larger American herbaria. The assemblage of this relatively large series of specimens afforded an excellent opportunity for a critical study of the group, and with the approval of Mr. Henderson the work was continued. The results are here recorded in the form of a preliminary revision of the genus.

[2] Kew Bull. Misc. Inf., p. 129. 1940.
[3] Gray in Proc. Am. Acad. **9**:198. 1874.
[4] *Ibid*. **19**:27. 1883.
[5] Rydberg in N. Am. Fl. **34**:44. 1914.
[6] Cabrera in Anal. Soc. Cient. Argent. **114**:187. 1932.
[7] See Gray, *loc. cit.*, and in Proc. Am. Acad. **15**:40. 1879.

Issued September 15, 1945.

from the closely related genus, *Bahia*, although Rydberg[8] places them in different subtribes. The fact that no or only a very few ligules are present in *Schkuhria*, whereas they are numerous in *Bahia*, is the most reliable though not natural character separating the two genera. When more specimens of *Bahia* are available this problem should be examined in greater detail. Although few generic transfers have been made in this paper, future study may warrant them. The "true" Schkuhrias are S. *pinnata* (Lam.) O. Kuntze and S. *anthemoidea* (DC.) Coult. and their varieties, while the remaining species of the genus are very closely related to certain species of *Bahia*, namely, *B. Bigelovii* Gray, *B. Schaffneri* S. Wats., *B. xylopoda* Greenm., and *B. Pringlei* Greenm.

Within the genus the pappus has always served as the chief diagnostic character, and Gray was one of the earliest to realize that it was "highly probable that the difference in the pappus, although constant in the specimens, is not of specific importance"[9]. However, the pappus, up to the time of Cabrera's[10] work, continued to serve as the main specific character. His realization that the pappus was extremely variable brought about a drastic reduction of the species then recognized in the South American flora. The writer has carried out his principles in the treatment of the North American species. In the present paper a total of six species, six varieties, and two forms are recognized.

Schkuhria is entirely American in its distribution with the exception of the few specimens reported from Africa which were probably introduced there. The genus extends in North America from the southwestern United States through Mexico and Guatemala, and in South America from Venezuela and Colombia to Argentina and Chile. Moreover, one species, S. *multiflora*, exhibits a discontinuous distribution between the two continents. It may well be that when more specimens are available for study many of the varieties and forms described herein would better be termed subspecies.

Schkuhria pinnata and its varieties, and perhaps S. *anthemoidea* to a lesser extent, have some use in popular medicine[11]. According to the labels on many of the specimens the plants are used as insect repellents or insecticides, particularly to kill fleas. *Schkuhria* deserves further investigation along these lines. An interesting observation made during this study was that the herbarium specimens of *Schkuhria* were quite free of insect damage.

The writer wishes to express his thanks to Dr. George T. Moore, Director, for the use of the facilities of the Missouri Botanical Garden; to Dr. Jesse More Greenman, for his helpful criticism and advice; to Miss Nell C. Horner and other members of the staff of the Missouri Botanical Garden, for their cooperation; to Sr. Angel L. Cabrera of the Museo de La Plata, Argentina; and to Mr. Norlan C.

[8] Rydb., *loc. cit.*, and p. 34.
[9] Gray in Smithson. Contr. Knowl. [Pl. Wright.] 5:95. 1853.
[10] Cabrera, *loc. cit.*
[11] O'Donnell and Rodríguez consider S. *pinnata* medicinally in "Las plantas medicinales del noroeste Argentina. II." Rev. Farm. (Buenos Aires) 84:149-159. 1942. I have not seen this reference.

Henderson, formerly graduate student, Henry Shaw School of Botany of Washington University. All opinions expressed, however, and any errors are those of the writer. Acknowledgments are made to the herbaria which have loaned specimens for examination. The abbreviations used in this paper are as follows: personal herbarium of A. L. Cabrera (C); Chicago Natural History Museum (formerly Field Museum of Natural History) (FM); Gray Herbarium of Harvard University (G); Missouri Botanical Garden (MBG); Philadelphia Academy of Natural Sciences (PA); University of Texas (T); United States National Herbarium (US).

Taxonomy

Schkuhria Roth, Cat. Bot. **1**:116. 1797; Benth. & Hook. Gen. Pl. **2**:403. 1873; Hoffm. in Engl. & Prantl, Nat. Pflanzenfam. 4^5:260. 1890; Cabrera, Comp. Bonaerenses, in Rev. Mus. La Plata, Secc. Bot. N. S. **4**:244. 1941, not *Schkuhria* Moench, Meth. 566. 1794.

Tetracarpum Moench, Meth. Suppl. 240. 1802; Rydb. in N. Am. Fl. **34**:44. 1914.
Mieria Llave in Llave & Lex. Nov. Veg. Desc. **2**:12. 1825.
Achyropappus Link & Otto, Ic. Pl. Rar. *pl. 30*. 1829, not *Achyropappus* HBK.
Hopkirkia DC. Prodr. **5**:660. 1836, not *Hopkirkia* Spreng.
Cephalobembix Rydb. in N. Am. Fl. **34**:46. 1914.

Slender, branching, erect or decumbent annuals, rarely perennials. Stems glabrate to hispid. Lowermost leaves opposite, upper alternate, pinnately or bipinnately divided into linear-filiform lobes, rarely simple, often impressed-punctate. Heads discoid or radiate with one to few rays. Involucre obconic to turbinate. Bracts of the involucre 4–18, obovate to oblanceolate, rather narrow, scarious and frequently colored on the margins, occasionally one or more smaller bracts present. Ray-flowers 1–3, rarely more, yellow to white, minute. Disk-flowers few to numerous, yellow, rarely red-tipped, lobes 5, extending about half the length of the throat, glandular tube equal to or less than the length of the throat. Style branches with short acute appendages. Achenes elongate, obpyramidal, generally 4-angled, villous or hispid on the angles, particularly at the base. Pappus of 8, rarely more, scarious squamellae, calloused at the base or with prominent mid-rib becoming an awn in some of the species.

Type species: *Schkuhria abrotanoides* Roth = **Schkuhria pinnata** var. **abrotanoides** (Roth) Cabrera.

KEY TO THE SPECIES, VARIETIES, AND FORMS OF SCHKUHRIA

A. Ligules lacking; achenes 10 or more.
B. Plants decumbent, annual; leaves inconspicuously punctate; involucral bracts persistently pubescent.
C. At least half of the pappus scales (squamellae) awned.
D. Awns of the pappus scales 1 mm. or longer; scales colorless ... 3. *S. multiflora*
DD. Awns less than 1 mm. long; scales maroon to purplish ... 4. *S. degenerica*
CC. Pappus scales not awned (except rarely 1 or 2).
D. Involucral bracts 5–6, 2 (rarely 3) mm. broad; achenes generally less than 15; squamellae rarely longer than 0.5 mm. ... 3a. *S. multiflora* var. *pusilla*

DD. Involucral bracts 7 or more, mostly 1–2 mm. broad; achenes generally more than 15; squamellae frequently longer than 0.5 mm. ..... 3. *S. multiflora*

BB. Plants erect, perennial; leaves conspicuously punctate; involucral bracts glabrate ..... 6. *S. Greenmanii*

AA. Ligule or ligules usually present; achenes 9 or less (except in *S. schkuhrioides*).

B. Ligules 1 (rarely 2 or lacking), 1–3 mm. long; involucral bracts obovate to lanceolate; squamellae over 0.5 mm. long (except in *S. pinnata* var. *virgata* f. *Pringlei*).

C. None of the pappus scales awned.

D. Scales about 2 mm. or longer, equal to subequal.

E. Achenes short-villous (hairs less than 0.4 mm. long) or hispid on the angles; ligule about 2 mm. long; pappus scales erose; South American plants ..... 1. *S. pinnata*

EE. Achenes long-villous (hairs more than 0.4 mm. long) on the angles; ligule about 1 mm. long; pappus scales merely denticulate; plants not of South America ..... 2d. *S. anthemoidea* var. *Wrightii*

DD. Scales about 1 mm. long, unequal, those on the angles longer ..... 1d. *S. pinnata* var. *virgata* f. *Pringlei*

CC. Half or more of the pappus scales awned.

D. Disk-flowers 5 or less (rarely 6); achenes long-villous on the angles.

E. Scales equal to subequal, generally more than half of them awned.

F. Ligule about 1 mm. long ..... 2. *S. anthemoidea*

FF. Ligule about 2 mm. long ..... 2a. *S. anthemoidea* var. *guatemalensis*

EE. Scales unequal, only those on the angles awned (rarely 1 or 2 of the intermediate scales awned).

F. Ligule about 1 mm. long ..... 2b. *S. anthemoidea* var. *Wislizenii*

FF. Ligule 2–3 mm. long ..... 2c. *S. anthemoidea* var. *Wislizenii* f. *flava*

DD. Disk-flowers 5–8; achenes short-villous on the angles.

E. All, or all but 1, of the scales awned; plants of South America ..... 1a. *S. pinnata* var. *octoaristata*

EE. About half of the scales awned.

F. Ligule 2 mm. long; pappus scales slightly overlapping; plants of South America ..... 1b. *S. pinnata* var. *abrotanoides*

FF. Ligule 1 mm. long; pappus scales not overlapping; plants of Mexico and Central America ..... 1c. *S. pinnata* var. *virgata*

BB. Ligules usually more than 2, 3–5 mm. long; involucral bracts broadly obovate; squamellae about 0.5 mm. long ..... 5. *S. schkuhrioides*

1. **Schkuhria pinnata** (Lam.) O. Kuntze, Rev. Gen. Pl. **3**:170. 1898, as synonymn of *Rothia pinnata;* Cabrera in Anal. Soc. Cient. Argent. **114**:187. 1932.

Pectis pinnata Lamarck in Jour. Hist. Nat. **2**:150. *pl. 31.* 1792.
S. bonariensis Hook. & Arn. in Hook. Jour. Bot. **3**:321. 1841, in part.
S. isopappa Benth. Plant. Hartweg. p. 205. 1845.
Amblyopappus mendocinus Philippi in Anal. Univ. Chile **36**:184. 1870.
S. coquimbana Philippi in Anal. Univ. Chile **90**:29. 1895.
Rothia pinnata α *pallida* O. Kuntze, *loc. cit.*, p. 170, in part.
R. pinnata β *purpurascens* O. Kuntze, *loc. cit.*, in part.
S. abrotanoides var. *pomasquiensis* Hieron. in Engl. Bot. Jahrb. **29**:53. 1900, in part.
S. advena Thellung in Fedde, Rep. Sp. Nov. **11**:308. 1912.

Erect annual; stems glabrate, striate, 20–50 cm. in height; leaves glabrate, pinnately or bipinnately dissected, or the upper and lower entire, linear, 10–40 mm. long, with the filiform segments 0.5–2 mm. wide, glandular-punctate; heads radiate, numerous, on peduncles 1–5 cm. long; involucre 4–5 mm. high, less wide; involucral bracts 4–5, obovate to oblanceolate, obtuse, punctate, with membranous margins, frequently colored purple, red, or yellow; disk-flowers 5–8, yellow, with tubular corollas; ligules 1, frequently glandular, pistillate, about 2 mm. long; achenes narrow, 3–4 mm. long, about three times as long as broad, hispid to short-villous on the angles, the hairs seldom longer than 0.3 mm.; pappus of 8 muticous scales, mostly unequal, irregular, erose.

Distribution: from Ecuador to Chile and Argentina, 3,000 to 10,000 ft.; elsewhere probably introduced.

ECUADOR—AZUAY: vicinity of Cuenca (from market), *Rose, Pachano & Rose 22829*, in part (G, US). IMBABURA: hills near Ibarra, *Jameson 675* (G, US). PICHINCHA: Andes, Cordillera de Quito, *Jameson 2* (US); "Guapulo prope Quito" (according to Bentham, *loc. cit.*), *Hartweg 1141* (fragment FM, G; photograph FM, G). TUNGURAHUA: vicinity of Ambato, *Pachano 59* (G), *s. n.* (NY, US). PROVINCE NOT DETERMINED: near Pomasqui, *Mille 474* in part (US).

BOLIVIA—COCHABAMBA: Pocona, *Steinbach 8655* (FM, G, MBG); Cereado (?), *Steinbach 9707* in part (FM, MBG); Cochabamba, *Cardenas 740* (US). LA PAZ: vicinity of Sorata, *Bang 1298* (G, MBG, NY, US). TARIJA: Bermejo and Tecumilla, *Fiebrig 2126* in part (G).

PERU—HUANUCO: Huanuco, coll. of 1778-88, *Ruiz & Pavon* in part (NY, US). JUNIN: Mito, *MacBride 3264* (G, US). DEPARTMENT NOT DETERMINED: Uspachaca, *MacBride & Featherstone 1294* (FM, G, US); valley of the Mantaro, *Weberbauer 6469* (US).

BRAZIL—SAO PAULO: Campinas, *Santoro 741* in part (US).

ARGENTINA—BUENOS AIRES: Pergamino, *Parodi 1385* (G). CATAMARCA: Andalgalá, *Jörgensen 1783* (G, MBG, US), *Cabrera 1015* (C). CORDOBA: Córdoba, Aug. 1878, *Hieronymus* (G), *Lossen 56* (G). LA RIOJA: Quachin, *Venturi 7844* (FM, G, MBG, US). JUJUY: Volcán, *Venturi 10192* (MBG), Rio Chico, *3412* (G). MENDOZA: Potrerillos, *Ragonese 250* (C). SANTA FE: Arroyo Seco, *Ragonese 252* (C). SAN JUAN: Quebrada de Zonada, *Rodrigo 2925* (C). SAN LUIS: between Merlo and Rincón, Santa Rosa, Feb. 1929, *Cabrera* (FM, US).

CHILE—COQUIMBO: Rivadavia: Río Turbio, *Cabrera 3502* (C). PROVINCE AND LOCALITY NOT DETERMINED: *Philippi 15425* (photograph, FM).

UNITED STATES. MASS.—NORFOLK CO.: Milton, 25 Sept. 1929, *Kidder*[12] (G, US).

MOZAMBIQUE—Lourenco Marques, *Morensen*[13] *18* (US).

1a. **Schkuhria pinnata** var. **octoaristata** (DC.) Cabrera in Anal. Soc. Cient. Argent. **114**:190. 1932.

S. octoaristata DC. Prodr. **5**:654. 1836.
S. pinnata β purpurascens O. Kuntze, Rev. Gen. Pl. **3**:170. 1898, in part.
S. abrotanoides var. *isopappa* Hieron. in Engl. Bot. Jahrb. **29**:53. 1900, in part.

Ligule 1–2 mm. long; squamellae of the achenes linear-lanceolate, equal, all or all but one of the scales gradually attenuated into an awn, exceeding the disk-corolla in length; otherwise as in the species.

[12] This plant was found growing in Kidder's garden as a weed. See Rhodora **31**:243. 1929. This specimen was determined as *S. Wrightii* but seems to be more closely related to *S. pinnata*.

[13] This plant seems to be introduced also. It is quite probable that *S. pinnata* var. *abrotanoides* occurs with the typical form here.

Distribution: Ecuador to northern Argentina, 2,000 to 10,000 ft.

ECUADOR—AZUAY: vicinity of Cuenca (from market), *Rose, Pachano & Rose 22829* in part (G, US). CHIMBORAZO: Riobamba, *Schimpff 922* in part (G). PROVINCE NOT DETERMINED: Andes, *Spruce 1789* (FM, G).

BOLIVIA—COCHABAMBA: Cordillera de Tunari, *Eyerdam 24661* (FM, MBG); Cereado (?), *Steinbach 9707* in part (G). LA PAZ: La Paz, *Aspland 4925* (US); vicinity of Sorata, *Mandon 71* (NY). SANTA CRUZ: Buenavista, *Steinbach 6937* (FM, G, MBG, NY, PA).

PERU—APURIMAC: Andahuailas, *Herrera 1492* (G). CUZCO: Cuzco, Feb. 1929, *Herrera* (FM). LIMA: Matucana, *MacBride & Featherstone 275* (FM, G, US).

ARGENTINA—JUJUY: Jujuy, Oct. 1892, *Kuntze* (US). SALTA: Salta, *Holmberg 10635* (MBG). SANTIAGO DEL ESTERO: Estancia el Remate, *Venturi 5836* (US). TUCUMAN: Dept. Burruyaca, *Venturi 2595* (US); Dept. Criancas, Capia, *Venturi 1082* (FM, MBG).

1b. **Schkuhria pinnata** var. **abrotanoides** (Roth) Cabrera in Anal. Soc. Cient. Argent. **114**:189. 1932.

S. abrotanoides Roth, Cat. Bot. 1:116. 1797; DC. Prodr. 5:654. 1836.
S. bonariensis Hook. & Arn. in Hook. Jour. Bot. 3:321. 1841, in part.
Rothia pinnata α *pallida* O. Kuntze, Rev. Gen. Pl. 3:170. 1898, in part.
S. abrotanoides var. *pomasquiensis* Hieron. in Engl. Bot. Jahrb. 29:53. 1900, in part.
S. abrotanoides var. *isopappa* Hieron. *loc. cit.*, in part.

Ligule about 2 mm. long; the squamellae on the angles awned, ovate-lanceolate, almost equalling the length of the disk-corolla; the intermediate scales shorter, unequal to subequal, muticous, slightly overlapping the scales on the angles, all of the squamellae strongly calloused at the base; otherwise as in the species.

Distribution: Venezuela and Colombia to Uruguay and Argentina, 750 to 12,000 ft.

VENEZUELA—PROVINCE NOT DETERMINED: San Rafael de Muchchies, *Pittier 13346* (NY, PA, US).

COLOMBIA—CUNDINAMARCA: Soacha near Bogotá, *Bros. Apollinaire & Arthur 109* (G, US). DEPARTMENT NOT DETERMINED: coll. of 1918, *Bro. Joseph* (US, NY), and 9 Aug. 1919, *Guatanita* (US).

ECUADOR—CHIMBORAZO: Riobamba, *Mille 474* in part (G); western Riobamba, *Schimpff 922* in part (MBG). PROVINCE NOT DETERMINED: Coquimba and Guayaquil, *Nee, s. n.* (FM).

BOLIVIA—CHUQUISACA: near Sucre, June 1943, *Hein*[14] (US). COCHABAMBA: vicinity Cochabamba, *Bang 755* (G, MBG, NY, US), *Buchtien 4803* (US). LA PAZ: Chimasi near Chulumani, *Buchtien 2442* (US), Millahuaya, *4802* (US). TARIJA: Bermejo and Tucumilla, *Fiebrig 2126* in part (G, US). DEPARTMENT NOT DETERMINED: Contana, *Buchtien 177* (FM, G, MBG, US); no locality given, *Bridges s. n.* (G).

PERU—AYACUCHO: Hunata, *Killip & Smith 23335* (NY, US); Prov. Cangallo, Hacienda Pajonal, *Stork & Horton 10793* (FM). CUZCO: Prov. Calca, Hacienda Urco, *Vargas 696* (FM). HUANUCO: Huanuco, coll. of 1778-79 in part, *Ruiz & Pavon* (FM, NY). LOCALITY NOT DETERMINED: *Klatt 654* (G), *Weberbauer 6449* (FM).

BRAZIL—SAO PAULO: Campinas, *Santoro 741* in part (US).

URUGUAY—No locality given, March 1876, *Loreato* (NY).

ARGENTINA—BUENOS AIRES: Pergamino, *Parodi 9581* (G, MBG). LA PLATA: Estacion Cargas, *Cabrera 7467* (C). CHACO: Villa Angela, *Boffa 1024* (C). CORDOBA: Río

[14] Note from Hein's herbarium label: "Piquipichana or flea-broom. The uses of this plant are to destroy fleas and to fight all sorts of disease, including malaria. It is taken as an infusion, stems and seed being poured in hot water; this same liquid serves to wet the floor of rooms that are to be disinfected."

Tercero, *Burkhart 10941* (G, MBG); south of Córdoba, coll. of 1878, *Hieronymus* (G); Córdoba, July 1891, *Kuntze* (NY, US), *Lossen 56* (FM, PA). SALTA: Dept. Rosario and Lerma, Campo Zuipano, *Venturi 8044* (US); Dept. Candelaria, Cerro de Chroville(?), *Venturi 3762* (G, US). SANTA FE: entre Rosacio y Casilda, *Ragonese 302* (C). SAN LUIS: Alto Pencoso, Feb. 1914, *Bruch & Carette* (C, G, US). MENDOZA: Santa Rosa, coll. of 1904-5, *Jensen* (US). TUCUMAN: Leales, *Venturi 7141 (714?)* (US).

FRANCE—cultivated in Paris, coll. of 1815, *Gay* (G).

The synonymy of the foregoing species and its two South American varieties is exceedingly involved. The writer has examined type material or photographs of types of many of these entities and finds it best to cite them at this time "in part" under more than one heading.

1c. **Schkuhria pinnata** var. **virgata** (Llave) Heiser, n. comb.

Mieria virgata Llave in Llave & Lex. Nov. Veg. Descr. **2**:12. 1825.
S. *virgata* DC. Prodr. **5**:654. 1836.
Tetracarpum virgatum Rydb. in N. Am. Fl. **34**:45. 1914.
Schkuhria glabrescens Gandoger in Bull. Soc. Bot. Fr. **65**:46. 1918.

Ligule about 1 mm. long; the squamellae of the pappus awned on the angles, lanceolate, shorter than the disk-corolla, the intermediate scales generally less than half as long, muticous, equal to subequal, not overlapping the other scales, only weakly calloused at the base; otherwise as in the species.

Distribution: northern Mexico to Guatemala, 5,000 to 9,000 ft.

MEXICO—AGUASCALIENTES: Rincón de Romos, *Shreve 9247* (G). CHIHUAHUA: Río Mayo, *Gentry 1926* (FM, G, MBG, US); near Guerrero, *Pringle 1292* in part (PA), Sierra Madre, Arroyo Ancho, *7082* (G, US). DURANGO: City of Durango and vicinity, *Palmer 509* (G, MBG, NY, US). FEDERAL DISTRICT: near Mexico, *Berlandier s. n.* (G, MBG); Mexico City, *Orcutt 4072* (FM, G, MBG); Lomas de Santa Fé, *Lyonnet 408* (MBG, NY, US); Tlalpam, *MacDaniels 46* (FM); Cerro de Guadalupe, *Pringle 8724* (G, MBG, NY, PA, US), vicinity of Mexico, *7928* (G, MBG, US). GUANAJUATO: Obregon, *Seler 1133* (G, NY, US). HIDALGO: hills above Pachuca, *Pringle 6943* (G, MBG, NY, PA, US); between Pachuca and Real del Monte, *Rose & Painter 6715* (NY, US); Real del Monte, *Ehrenberg 375a* (G). MEXICO: Valle de Mexico, *Bourgeau 372* (G, US); hills above Toluca, *Pringle 9096* (MBG, US); Temascaltepec, Mina de Agua, *Hinton 1405* (MBG, US), Pantoja, *6228* (G, NY). MICHOACAN: vicinity of Morelia, *Kenoyer A127* (FM). PUEBLA: vicinity of Puebla, *Arsène 352* (US), *2315* (MBG, NY, US), s. of Puebla, n. of Hacienda Batán, *1462* (US), Laguna de San Baetasar, 1 Aug. 1909 (US), and Cerro San Juan, 15 Aug. 1906 (US); Cerro del Corral de Piedra, near Oaxaca, *Purpus 3836* (FM, MBG, NY, US). SAN LUIS POTOSI: region of San Luis Potosi, *Parry & Palmer 427* in part (MBG, NY, PA, US); San Luis Potosi, *Schaffner 332 (750)* (NY, US). ZACATECAS: near Plateado (Plateros?), *Rose 2748* (US). LOCALITY NOT DETERMINED: *Coulter 314* (G, NY, PA), *Muller 1163* (NY), *Berlandier 708* (fragment FM), *Bonpland s. n.* (FM).

GUATEMALA—HUEHUETENANGO: Chacula, *Seler 2870* (G, US).

Schkuhria virgata DC. is best interpreted as a Central American variety of S. *pinnata*, for the only reliable difference lies in the nature of the pappus.

1d. **Schkuhria pinnata** var. **virgata** f. **Pringlei** (S. Wats.) Heiser, n. comb.

S. *Pringlei* S. Wats. in Proc. Am. Acad. **23**:278. 1888.
Tetracarpum Pringlei Rydb. in N. Am. Fl. **34**:44. 1914.

As the variety but the squamellae very short, less than 1 mm. long, those on the angles frequently somewhat awned, the intermediate scales still smaller, muticous.

Distribution: Chihuahua and Durango, Mexico.

MEXICO—DURANGO: along road from Durango to Santa Cruz, *Langman 2956* (PA). CHIHUAHUA: Majalca, *Le Sueur 1228* (FM), Cima (FM, T); southwestern Chihuahua, *Palmer 387* (NY, PA, US); base of Sierra Madre, *Pringle 1639* (MBG, NY), near Guerrero, *1292* in part (G, US).

It is worthy of note that in South America certain specimens of S. *pinnata* and its variety *abrotanoides* occur which have a pappus almost identical with that of the above form. I have not separated these specimens (*Venturi 8044* in part, *Steinbach 8655* in part) from the variety, but it is interesting to observe their parallel development. S. *anthemoidea* var. *Wrightii* in Texas also occurs with a very reduced pappus which resembles this form somewhat. I choose to regard S. *Pringlei* S. Wats. simply as a form of S. *pinnata* var. *virgata*, for the only constant difference is found in the size of the squamellae.

2. **Schkuhria anthemoidea** (DC.) Coult. in Donn.-Smith, Enum. Pl. Guat. 4:93. 1895, in part, as "*anthemoides*" sphalm.

Hopkirkia anthemoidea DC. Prodr. 5:660. 1836.
S. *Hopkirkia* Gray in Smithson.Contr. Knowl. [Pl. Wright] 5:94. 1853.
Tetracarpum anthemoideum Rydb. in N. Am. Fl. 34:45. 1914.

Erect annual; stems glabrate, striate, 20–50 cm. in height; leaves glabrate, pinnately or bipinnately dissected into linear filiform segments, 10–40 mm. long, 0.5–2 mm. wide, or the upper and lower entire, conspicuously glandular-punctate; heads radiate, numerous, on peduncles 1–5 cm. long; involucre 5–7 mm. high, less wide; involucral bracts 4–5, obovate, obtuse, glabrous, punctate, green with scarious colored margins, deep purple to red; disk-corollas rarely more than 5, yellow, rarely red-lobed; ligule 1, pistillate, about 1 mm. long; achenes 3–4 mm. long, about twice as long as broad, thick, striate, densely villous on the 4 angles, the hairs 0.4 mm. or longer; squamellae equal to subequal, ovate-lanceolate to lanceolate, most or all of the scales awn-tipped, about the length of the disk-corolla or only slightly exceeding it.

Distribution: Arizona to southern Mexico, 2,000 to 8,000 ft.

UNITED STATES—ARIZONA: COCHISE CO.: Chiricahua Mts., top of main ridge between Rock and Turkey creeks, *Blumer 1635* in part (G, NY); n. of Fort Huachuca, *Lemmon 4774* (G); Huachuca Mts., *Lemmon 2770* (US); Sunnyside, *Kearney & Peebles 13834* (US).

MEXICO—CHIAPAS: between Tuxtla and San Cristobal, *Nelson 3122* (US). CHIHUAHUA: Río Mayo, Cerro Quicorichi, *Gentry 1924* (FM, G, MBG, US); near Chihuahua, *Pringle 772* (G, MBG, NY, PA, US), hills around Parral, *13566* (G, US). COLIMA: Alzada, *Orcutt 4625* (FM, MBG). FEDERAL DISTRICT: Olivar, *Orcutt 3683* (FM); pyramid of Cuicuilco, Tlalpam, *McDaniels 710* (FM); Tlalpam, *Seler 4111* (G, US); hills north of Mexico City, *Pringle 6781* (G, MBG, NY, PA, US), Cerro de Guadalupe, *0057* (NY). GUANAJUATO: valley of Silao, 24 kilo. s. of Guanajuato, Nov.-Dec. 1893, *Duges* (G). GUERRERO: Tasco, *Abbott 447* (G). JALISCO: Lake Chapala, *Lemmon 76* (G); Tequila, *Palmer 365* (G, MBG, NY, PA, US); hills above Etzatlan, *Pringle*

11568 (G, US). MEXICO: Molino, *McDaniels 587* (FM), *Rose & Painter 6979* (FM, MBG, NY, US); near Cuernavaca, *McDaniels 333* (FM), *Rose & Hough 4445* (US). NAYARIT: Cerro de la Cruz, e. of Tepic, *Ynes Mexia 657* (G, FM, MBG, NY, US). OAXACA: coll. of 1922, *Reko*[15] (US). PUEBLA: vicinity of Puebla, Cerro Guadalupe, *Arsène 1869* (MBG, US), Fort Guadalupe, *86* (US), Cerro and Fort Guadalupe, *1198* (US). SONORA: Cañon de Aribabi, south of Aribabi, *White 2747A* (G). VERA CRUZ: Orizaba, *Miller 270* (US); Corral de Piedras, *Purpus 8241* (G, MBG, NY, US). STATE NOT DETERMINED: *Haenke* (photograph of TYPE, FM).

2a. **Schkuhria anthemoidea** var. **guatemalensis** (Rydb.) Heiser, n. comb.

S. virgata Hemsl. Biol. Centr.-Am. Bot. **2**:212. 1881, in part.
S. anthemoides Coult. in Donn.-Smith, Enum. Pl. Guat. **4**:93. 1895, in part.
Tetracarpum guatemalense Rydb. in N. Am. Fl. **34**:45. 1914.
S. guatemalensis Standl. & Steyermark in Field Mus. Publ. Bot. **22**:319. 1940.

Involucral bracts generally reddish or purplish at the apex; ligule about 2 mm. long; disk-flowers 4–6; squamellae subequal, mostly ovate-lanceolate, 4–7 of the scales awned, equalling or slightly shorter than the disk-corolla, always strongly calloused at the base; otherwise as in the species.

Distribution: Guatemala and El Salvador, 1,500 to 8,000 ft.

GUATEMALA—AMATITLAN: Canchalagua, Laguna, *Ruano 1294* (FM); Canchalagua, *Morales 792* (US). CHIQUIMULA: llanos around Ipala, *Steyermark 30316* (FM, NY). GUATEMALA: Estancia Grande, *Standley 59186* (FM, NY); Finca Bretana, road between Guatemala and Fiscal, *Standley 59757* (FM). HUEHUETENAGO: Aguacatan road, 10 km. e. of Huehuetenango, *Standley 82115* (FM); no locality given, *Skutch 1589* (G). JALAPA: Laguna de Ayarza, *Heyde & Lux 3802* (FM, G, MBG, NY, US); between Jalapa and base of Volcán Jumay, *Steyermark 32259* (FM). JUTIAPA: n. of Jutiapa, *Standley 60512* (FM). DEPARTMENT NOT DETERMINED: "La Aurora," *Ruano 568* (US); no locality given, *Tonduz 885* (G, NY, US).

EL SALVADOR—AHUACHAPAN: *Padilla 235* (MBG, NY, US). SANTA ANA: near Chalchuapa, *Calderón 962* (FM, MBG, NY, US). DEPARTMENT NOT DETERMINED: *Renson 305* (FM, G, NY, US); La Cebadilla, *Calderón 1236* (G, US).

I can regard this plant only as a variety of *S. anthemoidea*, from which it can be distinguished only with difficulty if the locality of the collection were unknown.

2b. **Schkuhria anthemoidea** var. **Wislizenii** (Gray) Heiser, n. comb.

S. Wislizeni Gray in Mem. Am. Acad. II. **4**:96. 1849.
Tetracarpum Wislizeni Rydb. in N. Am. Fl. **34**:45. 1914.

Involucral bracts yellow to purple at the apex; ligule seldom over 1 mm. long, almost as wide; squamellae lanceolate to ovate-lanceolate, those on the angles awn-tipped, the intermediate ones shorter, muticous.

Distribution: Arizona to central Mexico, 5,000 to 8,000 ft.

UNITED STATES. ARIZONA—COCHISE CO.: Chiricahua Mts., top of main ridge between Rock and Turkey creeks, *Blumer 1634* (FM, G, MBG, NY, US); Mule Mts., *Harrison & Kearney 6224* (G, US).

MEXICO—CHIHUAHUA: Mojarachic, *Knobloch 5464* (FM), southwestern Chihuahua, *Palmer 387* (G); hills about Parral, *Pringle 13567* (G, US); Cosihuinachic Mts., *Wis-*

[15] Note from herbarium label: "Brooms of plants sold in markets, used for exterminating fleas."

lizenus 195 (TYPE COLLECTION G, MBG). FEDERAL DISTRICT: Cerro de Guadalupe, *Pringle 9957* (G, MBG, US). HIDALGO: Pachuca, *Orcutt 3921* (FM, G, MBG).

2c. **Schkuhria anthemoidea** var. **Wislizenii** f. **flava** (Rydb.) Heiser, n. comb.

Tetracarpum flavum Rydb. in N. Am. Fl. 34:46. 1914.

As the variety but the ligule 2–3 mm. long, less broad.

Distribution: southern Mexico. (I have examined this form only from Oaxaca at altitudes from 5,000 to 7,500 ft.)

MEXICO—OAXACA: District of Etla, Las Sedas, *Conzatti 5004* (MBG); Oaxaca, *Galeotti 2049 (2045?)* (G); Reyes, *Nelson 1710* (US); limestone hills near Etla, *Pringle 4881* (G, MBG, NY, PA, US); Sierra de San Felipe, *Smith 263 & 626* (TYPE COLLECTION MBG, US).

This species of Rydberg's is no more than a form differing from the variety only in the length of the ligule.

2d. **Schkuhria anthemoidea** var. **Wrightii** (Gray) Heiser, n. comb.

S. *Wrightii* Gray in Smithson. Contr. Knowl. [Pl. Wright.] 5:95. 1853.
Tetracarpum Wrightii Rydb. in N. Am. Fl. 34:44. 1914.

Ligule about 1 mm. long; disk-flowers 5 or less; squamellae obovate, subequal, rounded at the apex, denticulate; otherwise as in the species.

Distribution: southwestern United States and northwestern Mexico, 3,500 to 7,500 ft.

UNITED STATES. ARIZONA—COCHISE CO.: Chiricahua Mts., top of main ridge between Rock and Turkey creeks, *Blumer 1635* in part (MBG, NY, US); Silver Creek, Chiricahua National Park, *Eggleston 10035* (G, US); Mule Mts., *Harrison & Kearney 6088* (G); Ramsey Canyon, Huachuca Mts., *Jones 25041* (G), and 29 Sept. 1929 (MBG); Apache Pass, Chiricahua Mts., Sept. 1881, *Lemmon* (MBG); plain near Ft. Huachuca, *Peebles, Harrison & Kearney 3468* in part (US); base of the Huachuca Mts., 15 Sept. 1884, *Pringle* (G, NY, PA, US); near Fort Huachuca, *Wilcox 334* (NY, US). PIMA CO.: Greaterville, *Shreve 4073* (MBG). SANTA CRUZ CO.: Sonoita, *Harrison & Kearney 5703* (US). NEW MEXICO—DONA ANA CO.: Organ Mts., 4 Sept. 1898, *Cockerell* (US), *Wooton 445* (G, MBG, NY, US), 28 Sept. 1902 (MBG), and 20 Sept. 1908, *Wooton & Standley* (US). GRANT CO.: (?) near Santa Rita de Cobre, 21 Sept. 1880, *Greene* (MBG, NY). SIERRA CO: Fruijilla Creek, *Metcalfe 1358* (G, MBG, NY, US); Lake Valley, coll. of 1916, *Beals* (US). TEXAS—JEFF DAVIS CO.: Davis Mts. near Mt. Locke, *Hinckley 478* (FM, NY), Mt. Livermore, 28 Sept. 1935 (FM, T); Davis Mts., *Palmer 30652a* (MBG, PA, T), 19 Sept. 1918, *Young* (T); Limpia Cañon, *Nealley 189* (FM); 5 mi. n. w. of McDonald Observatory, *Innes & Moon 1133* (G); Ft. Davis, 23 Aug. 1941, *Strandtmann* (T).

MEXICO—CHIHUAHUA: San Diego Cañon, Sierra Madre Mts., 16 Sept. 1903, *Jones* (NY); 30 mi. s. w. of Chihuahua, *Muller 3341* (G); vicinity of Chihuahua, *Palmer 346* (US); rocky hills near Chihuahua, *Pringle 607* (G, MBG, NY, PA, US), *772* in part (PA), hills near Chihuahua, *974* (MBG, NY), mesas near Carretas, *2001* (G, US), dry hills, Parral, *10113* (G, MBG, NY, PA, US); eastern Chihuahua, just east of Organos, *Stewart & Johnston 2015* (G); Santa Eulalia, 30 Sept. 1885, *Wilkenson* (US). SONORA: San Pedro, *Hartman 854* (G); no locality given, *Wright 1254* (TYPE COLLECTION G, PA).

3. **Schkuhria multiflora** Hook. & Arn. in Hook. Jour. Bot. 3:332. 1841.

Achyropappus schkuhrioides Don. ex Hook. & Arn., *loc. cit.*, not *Achyropappus*

schkuhrioides Link & Otto.
S. *Neo-Mexicana* Gray in Mem. Am. Acad. II. 4:96. 1849.
Amblyopappus Neo-Mexicanus Gray in Torr. Pacif. R. R. Rept. 4:106. 1857.
Bahia Neo-Mexicana Gray in Proc. Am. Acad. 19:27. 1883.
Bahia Gilliesii Gray, *loc. cit.*, p. 28.
S. *pusilla* var. *aristata* R. E. Fries in Nova Acta Soc. Sci. Upsal. IV. 11:85. *t. 6, 8,* 1905.
Achyropappus neo-mexicanus Rydb. Fl. Colo. 377. 1906.
Cephalobembix neo-mexicana Rydb. in N. Am. Fl. 34:46. 1914.
S. *pusilla* var. *longepedicellata* Hauman in Anal. Soc. Cient. Argent. 86:328. 1918.
S. *multiflora* var. *aristata* Cabrera in Anal. Soc. Cient. Argent. 114:193. 1932, as "*multflora*" sphalm.

Annual, more or less decumbent, 5–25 cm. in height; stems short-glandular-hairy to glabrate; leaves pinnately dissected into lobes 0.5–1 mm. wide, up to 3 cm. long; petioles 0.2–1 cm. long; peduncles glandular-pubescent, 0.5–3 cm. long; heads discoid; involucres turbinate to obconic, 5–10 mm. wide, 5–6 mm. high; bracts of the involucre 7–9, green, scarious-tipped, frequently red or yellow on the margins, 1–2 mm. wide and narrowing gradually; disk-corollas 15–30, yellow, occasionally red-tipped; achenes black with a few scattered hairs on the faces, white-villous on the 4 angles, 3–4 mm. long; squamellae 1–2 mm. long, obtuse to acutish, rarely several or all of the squamellae awned, usually strongly calloused at the base.

Distribution: southwestern United States into northern Mexico in North America and Bolivia to Argentina and Chile in South America, 5,000 to 11,000 ft.

UNITED STATES. ARIZONA—NAVAJO CO.: between Kayenta and Betatakin, *Eastwood & Howell 6574* (FM). YAVAPAI CO.: Prescott, *Griffiths 7349* (MBG). COLORADO—HUERFANO CO.: Huerfano, *Parry 124, 125* (MBG). RIO GRANDE CO.: banks of the Rio Grande near Del Norte, *Brandegee 1228* (MBG), 12 mi. below Del Norte on the banks of the Rio Grande, *4248* (MBG). NEW MEXICO—LINCOLN CO.: White Mts., *Wooton 297* (MBG). SAN MIGUEL CO.: near Pecos, *Standley 5052* (MBG). SANTA FE CO.: Santa Fe, *Fendler 416* (MBG), *Mulford 1366* (MBG); southeast of Santa Fe, 9 Sept. 1881, *Engelmann* (MBG); no locality given, *Brandegee 12070* (MBG). SOCORRO CO.: Mogollon Mts., on or near west fork of Gila R., *Metcalfe 580* (MBG). VALENCIA CO.: Cubero, *Rusby 706* (PA). TEXAS—BREWSTER CO.: Chisos Mts., *Mueller 8232* (FM, G, MBG, NY, T, US).

MEXICO—CHIHUAHUA: Potrero Mts., *Pringle 773* (MBG); Majalca, *LeSueur 1229* (FM, MBG); near Colonia Garcia in the Sierra Madre, *Townsend & Barber 286* (MBG).

BOLIVIA—LA PAZ: La Paz, *Buchtien 4802, 9227* (G, MBG).

PERU—AREQUIPA: Arequipa, *Pennell 13051, 13162* (FM, G, US). MOQUEGUA: Torata, *Weberbauer 7408* (US).

ARGENTINA—MENDOZA: Potrerillos, *Ragonese 248* (C). TUCUMAN: Valle de Tafí, coll. of 1908, *Bruch* (C, US).

CHILE—ATACAMA: Dept. Vallenar, Rio de la Laguna Grande, above the mouth of Rio Lag. Chica, *Johnston 5889* (G, US). PROVINCE NOT DETERMINED: (Chilecito?), *Gillies s. n.* in part (G).

Notwithstanding the discontinuous distribution of S. *multiflora* and S. *neo-mexicana* the two appear to be the same species. This species appears to be very closely related to certain species of *Bahia* but lacks the rays typical of that genus. For this type of distribution see I. M. Johnston, Jour. Arn. Arb. **21**:336. 1940.

The problem of the relationship between the aristate and the non-aristate specimens of this species can only be more clearly determined when more speci-

mens are available for study. *Buchtien 4802, 9227* in part, *Weberbauer 7408* in part, and *Gillies s. n.* in part, have the pappus provided with four or more awns. In a letter to the author Cabrera writes: "The forms with aristas may be possibly included under the name S. *multiflora* var. *typica* as the original diagnosis gives four mutic and four aristate paleae." Owing to the apparently continuous variation it is probably best at present to include the aristate forms with the species. It is worthy of note to point out that so far no North American specimens of S. *multiflora* have been found with aristate squamellae.

3a. **Schkuhria multiflora** var. **pusilla** (Wedd.) Cabrera in Anal. Soc. Cient. Argent. **114**:192. 1932.

S. *pusilla* Wedd. Chloris Andina, p. 17, *t. 14, B.* 1855.
Rothia pusilla O. Kuntze, Rev. Gen. Pl. 3:170. 1898.
S. *pusilla* var. *longepedicellata* Hauman in Anal. Soc. Cient. Argent. 86:328. 1918, in part.

Annual, 1–10 cm. in height; petioles rarely longer than 5 mm.; peduncles 0.2–0.5 cm. long; involucre about 5 mm. high and rarely wider; involucral bracts 5–6, 1–3 mm. wide, olive-green, frequently with yellow (rarely purple) scarious edges; disk-corollas 10–20; achenes about 3 mm. long; squamellae mostly obtuse, 0.5–1 mm. long; otherwise as in the species.

Distribution: Bolivia to Argentina, 8,000 to 12,000 ft. The variety appears to grow at slightly higher altitudes than the species, perhaps accounting in part for its smaller size.

Bolivia—la paz: La Paz, *Buchtien 3069* (US), Cerro de Calvoirio, *707* (US). potosi: no locality given, *Cardenas 433* (US). province and locality not determined: *Mandon 73* (FM, NY).

Peru—puno: vicinity of Lake Titicaca, *Shepard 41* (G, NY, US); Chuquibambilla, *Pennell 13364* (FM, PA).

Argentina—catamarca: Dept. of Andalgalá, El Candado, *Jörgensen 1282* (G, MBG, US). salta: El Alisal, Cerro del Cajón, *Rodriguez 1422* (C). tucuman: Dept. of Chicligasta, *Venturi 3298* (US).

Country and Locality not Determined: *Weddell 4415* (FM).

I have not seen the type of *Rothia intermedia* of Kuntze, but it is quite probable that it may fall into synonymy under this variety.

4. **Schkuhria degenerica** (O. Kuntze) R. E. Fries, Arkiv för Bot. **5**[13]:22. 1906.

S. *pusilla* var. *major* Schz. Bip. in Bull. Soc. Bot. Fr. 7:80. 1865; Linnaea 34:529. 1866, *nomen nudum.*
S. *oolepsis* Schz. Bip. *loc. cit., nomen nudum.*
Rothia degenerica O. Kuntze, Rev. Gen. Pl. 3:169. 1893.

Decumbent annual, 10–40 cm. in height; stem lightly glandular-villous; leaves alternate, pinnately or bipinnately divided into divisions about 1 cm. wide; heads discoid on peduncles 1.0–1.5 cm. long; involucre turbinate, 7–9 mm. long, 1 cm. or less wide; involucral bracts 5–7, green, hispidulous, with a scarious yellow, rarely purplish, apex; achenes 30–40, narrow, weakly 4-angled, more or

less compressed, short-villous on the angles, more densely so at the base, 3–4 mm. long; squamellae 2.0–3.5 mm. long, about 1 mm. wide, slightly exceeding the disk-corolla in length, erose, with a reddish-maroon midrib projected into an awn in about one-half of the squamellae.

Distribution: known only from Bolivia.

BOLIVIA—COCHABAMBA: Cochabamba, *Bang 966* (FM, G, MBG, PA, US). LA PAZ: Prov. Larecaja, San Pedro near Sorata, *Mandon 72* (G, FM).

5. **Schkuhria schkuhrioides** (Link & Otto) Thellung in Fedde, Rep. Sp. Nov. **11**:308. 1912.

Achyropappus schkuhrioides Link & Otto, Ic. Pl. Rar., p. 59, *pl. 30*. 1829, not *Achyropappus schkuhrioides* Don. ex. Hook. & Arn.
S. *senecioides* Nees, Del. Sem. Hort. Bot. Bonn. 1831.
Bahia schkuhrioides Gray in Proc. Am. Acad. **19**:27. 1883.
Tetracarpum schkuhrioides Rydb. in N. Am. Fl. **34**:46. 1914.

Erect annual, 40–80 cm. in height; stem striate, grooved, glabrate; leaves pinnately dissected into narrow linear divisions, 3–7 cm. long, punctate; heads radiate on peduncles 2–5 cm. long; involucral bracts 6–8, obovate to ovate, with yellow scarious tips, frequently subtended by 1 or more smaller bracts; ligules 1–4, obovate-cuneate, 3–5 mm. long; disk-corollas 15–20, yellow with glandular tubes; achenes elongate-obpyramidal, about 3–4 mm. long, with a few short hairs on the angles; squamellae obovate, about 0.5 mm. long.

Distribution: central and southern Mexico.

MEXICO—DURANGO: Durango, *Palmer 576* (MBG, NY). MEXICO: vicinity of Mexico, *Pringle 9855* (MBG, NY). MICHOACAN: vicinity of Morelia, *Arsène 5723* in part (G), Loma Santa Maria, *5837* (FM, G, MBG, NY, US), Lieux (?) in Andes, *3127* (MBG, US); vicinity of Lerma, north of La Piedad, *Pringle 3281* (MBG, NY).

This species is a connecting link with *Bahia*. The glandular-punctate leaves and bracts and the small number of ligules have led me to retain it in *Schkuhria*. The pappus most nearly resembles that of S. *pinnata* var. *virgata* f. *Pringlei*, but on the basis of the appearance and number of achenes S. *schkuhrioides* is more closely related to S. *multiflora*.

6. **Schkuhria Greenmanii** Heiser, n. sp.

Herba perennis, 35–65 cm. alta; caulibus glandulari-punctatis; foliis alternis, pinnato-dissectis, raro simplicibus, segmentis linearibus vel filiformibus, obtusis, impresso-punctatis, 2–7 cm. longis; capitulis homogamis, 1 cm. altis, usque ad 0.5 cm. latis; involucri bracteis 4–5, obovatis apice obtusissimis, marginibus scariosis et fimbrillatis; ligulis nullis; disci floribus 10–20, corollis 5-dentatis, 2–4 mm. longis; achaeniis ca. 3 mm. longis, sparse hirsutis vel ad angulos adpresso-pubescentibus; pappi paleis plerumque 8, 3 mm. longis, lanceolatis, dentatis, aristulatis, 1-nerviis, nervo-medio prominente.

Erect perennial, 35–65 cm. in height; stems striate, glabrate, glandular-punctate; leaves mostly alternate, pinnately dissected into linear-filiform divisions, conspicuously glandular-dotted, rarely entire; heads discoid on peduncles 2–5 cm. long; involucre about 1 cm. high, less wide, turbinate, bracts of the involucre 4–5,

more or less keeled at the base, obovate, margins scarious and provided with a fringe; disk-flowers 10–20 with yellow corolla and glandular tube; achenes 4-angled, lightly hirsute on the angles, more so at the base, about 3 mm. long; squamellae usually 8 (7–10), lanceolate, erose on the margins, provided with a conspicuous midrib extending into an awn, 3 mm. long, almost equalling the length of disk-corolla.

MEXICO—MEXICO: District of Temascaptepec, Luvianos, *Hinton 4507* (MBG TYPE; co-types at G, NY, US).

This plant is the only perennial *Schkuhria* known, and on the basis of the pappus seems most closely related to S. *anthemoidea*, under which name it was originally determined. It also has certain affinities with *Bahia*, from which it is distinct by the lack of ray-flowers.

EXCLUDED NAMES AND SPECIES

Schkuhria anthemoides Wedd. ex Hook. & Jacks. Ind. Kew. **4**:827. 1895, as synonym = **Achyropappus anthemoides** HBK. Nov. Gen. & Sp. **4**:259. 1820, not S. *anthemoides* of Coult.

S. *Bigelovii* Gray in Proc. Am. Acad. **9**:199. 1874. = **Bahia Bigelovii** Gray in Torr. Bot. Mex. Bound. p. 96. 1859. This species is probably best retained in *Bahia* for the present. It is closely related to S *multiflora*.

S. *biternata* Gray in Proc. Am. Acad. **9**:199. 1874. = **Bahia biternata** Gray in Smithson. Contr. Knowl. [Pl. Wright.] **5**:95. 1853.

S. *glomerata* Rob. & Seat. in Proc. Am. Acad. **28**:109. 1893 = **Florestina pedata** (Cav.) Cass. in Dict. Sci. Nat., Planch. Bot. Dicot. **61**:*t.86*. 1816-29.

S. *integrifolia* Gray in Am. Nat. **8**:213. 1874; *Bahia nudicaulis* Gray in Proc. Am. Acad. **19**:27. 1883; *Bahia integrifolia* Macbr. in Contr. Gray Herb. **56**:39. 1918. = **Platyschkuhria integrifolia** Rydb. in Bull. Torr. Bot. Club **33**:155. 1906.

S. *pedata* Gray in Proc. Am. Acad. **9**:199. 1874. = **Bahia pedata** Gray in Smithson. Contr. Knowl. [Pl. Wright.] **3**:123. 1852.

S. *platyphylla* Rob. & Greenm. in Am. Jour. Sci. **50**:156. 1895. = **Florestina platyphylla** Rob. & Greenm. in Proc. Am. Acad. **32**:49. 1896.

Schkuhuria Schiedei Gandoger in Bull. Soc. Bot. Fr. **65**:46. 1918. I have not seen a specimen of this plant, but from Gandoger's very scanty description it may not even be a *Schkuhria*. I can not recognize this species.

S. *viscossima* Standl. & Steyerm. in Field Mus. Publ. Bot. **22**:318. 1940 = **Florestina viscossima** (Standl. & Steyerm.) Heiser, n. comb. The affinities of this plant are with *F. pedata* (Cav.) Cass., and the nature of the style clearly places it in the genus *Florestina*.

S. *Woodhousei* Gray in Proc. Am. Acad. **19**:199. 1874; *Picradeniopsis Woodhousei* Rydb. in Bull. Torr. Bot. Club **37**:333. 1910. = **Bahia Woodhousei** Gray in Proc. Am. Acad. **19**:28. 1883.

MULTI-DIMENSIONAL GRAPHICAL REPRESENTATION FOR ANALYZING VARIATION IN QUANTITATIVE CHARACTERS

ELLEN MARIE KERN

Heermans Scholar, Henry Shaw School of Botany of Washington University

AND CHARLOTTE ALPER

A basic difficulty in analyzing data on species crosses is that the mind is ordinarily incapable of comprehending the simultaneous occurrence of variation in several different characters. In studying the results of a cross between *Nicotiana Langsdorfii* and *N. alata*, the authors developed a graphical method for analyzing the variation in four distinct characters considered together.

The four characters used are all measured in the flower, for, as East pointed out in a classic paper on the subject[1], floral dimensions are better than vegetative

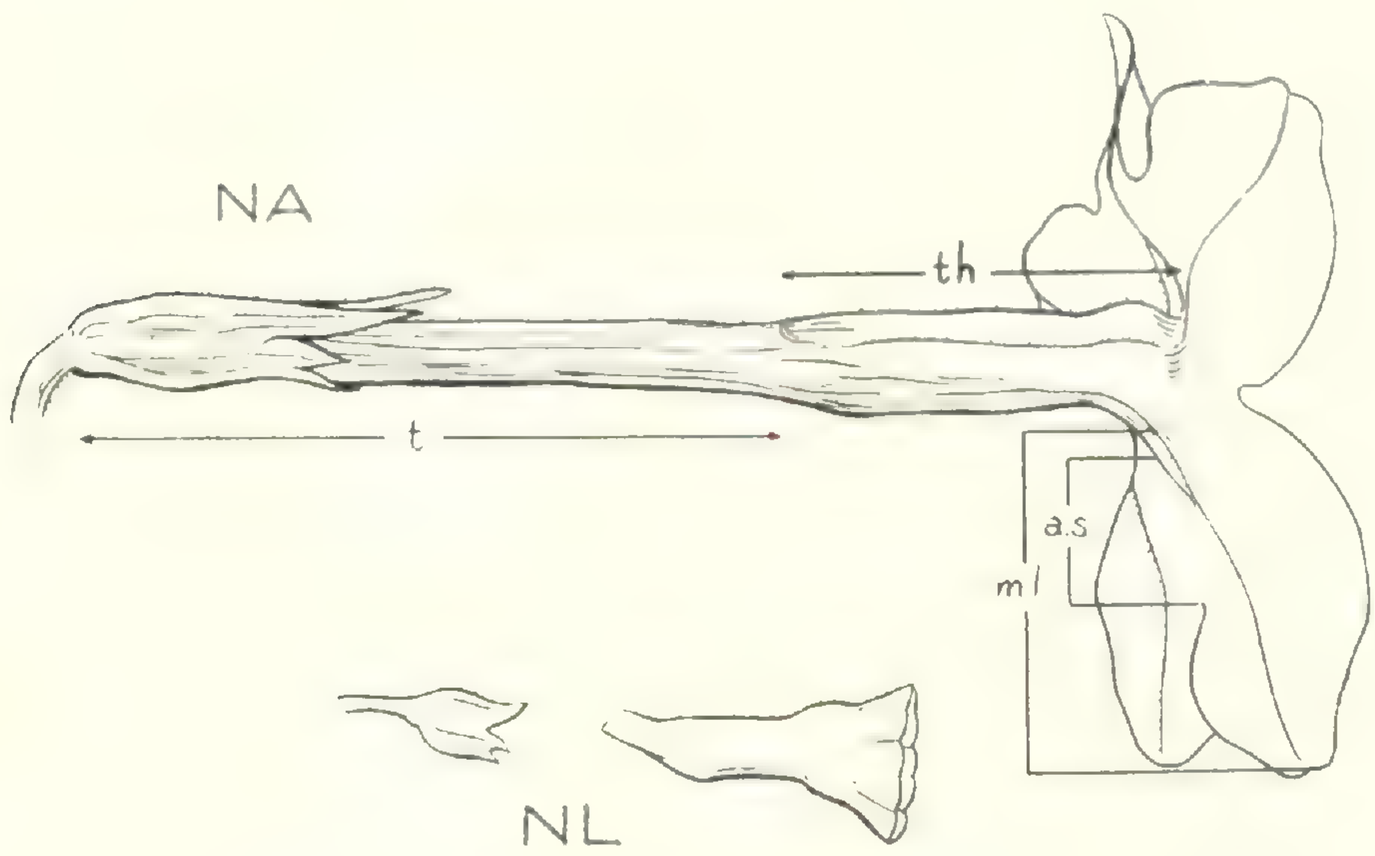

Fig. 1. Flowers of *Nicotiana alata* (NA) and *N. Langsdorfii* (NL): *t* — tube, *th* — throat, *ml* — maximum lobe, *as* — adjacent sinus.

parts for studies of so-called quantitative characters and measurements. These four characters are: the length of throat, maximum lobe, adjacent sinus[2], and tube (fig. 1), each one being expressed by a different dimension of a fourth-dimensional figure. Thus, the length of the throat is the length of the horizontal, or first dimension; maximum lobe, the vertical or second dimension; adjacent sinus, the third dimension; and tube, the fourth. A fourth-dimensional picture of each generation—the two parents, *Nicotiana Langsdorfii* (NL) and *N. alata* (NA), the F-1 generation (NLA), and two sister families of the F-2 (NLALA-b

[1] East, E. M. Significant accuracy in recording genetic data. Amer. Jour. Bot. **3**:211-222. 1916.
[2] Smith, Harold H. The relation between genes affecting size and color in certain species of *Nicotiana*. Genetics **22**:361-375. 1937.

and NLALA-g)—is obtained by using the group's median measurements of each character. To permit accurate comparison, the same scales and angles of slope are used throughout.

As may be seen in fig. 2, extreme dissimilarities in size and shape occur in the parent species, NL and NA, which differ significantly in all six ratios. The greatest difference in proportions is due to the throat being the longest absolute dimension in NL, and the tube longest in NA.

The F-1 and F-2 generations occupy an intermediate position between NL and NA, with the F-1 exhibiting the effects of hybrid vigor by its slightly larger size (very nearly proportionately so) than the F-2. The intermediate condition of the F-1's and F-2's is further emphasized by the approximately equal lengths of tube and throat. The individuals of any F-2 between two very unlike parents will be segregating for various characters which will affect survival differentially, even under experimental conditions. There will be susceptibility to various diseases and environmental conditions—in this case wilt and mosaic, crowding in the seed-pan, survival during cloudy weather in short days of winter, etc. Besides supplying a means for comparing the F-1 and F-2 generations, this graphical method permits the comparison of the F-2's in successive years to obtain an average.

This multi-dimensional representation not only allows the mind to conceive the effects of more than one character at a time, but also permits analysis of just those characters considered, excluding those characters which may, in analyses using the actual flower, cause optical illusions and distracting effects. Additional and more detailed comparisons may be made from the figures—such as variation in actual size of one particular character, the effect of similarity in size of two or three characters, the combined effect of variation in two, three, or four characters. From this treatment of the data it is also obvious that more than four dimensions (or characters) may be considered simultaneously.

The authors wish to thank Dr. Edgar Anderson for many helpful suggestions and the use of his data.

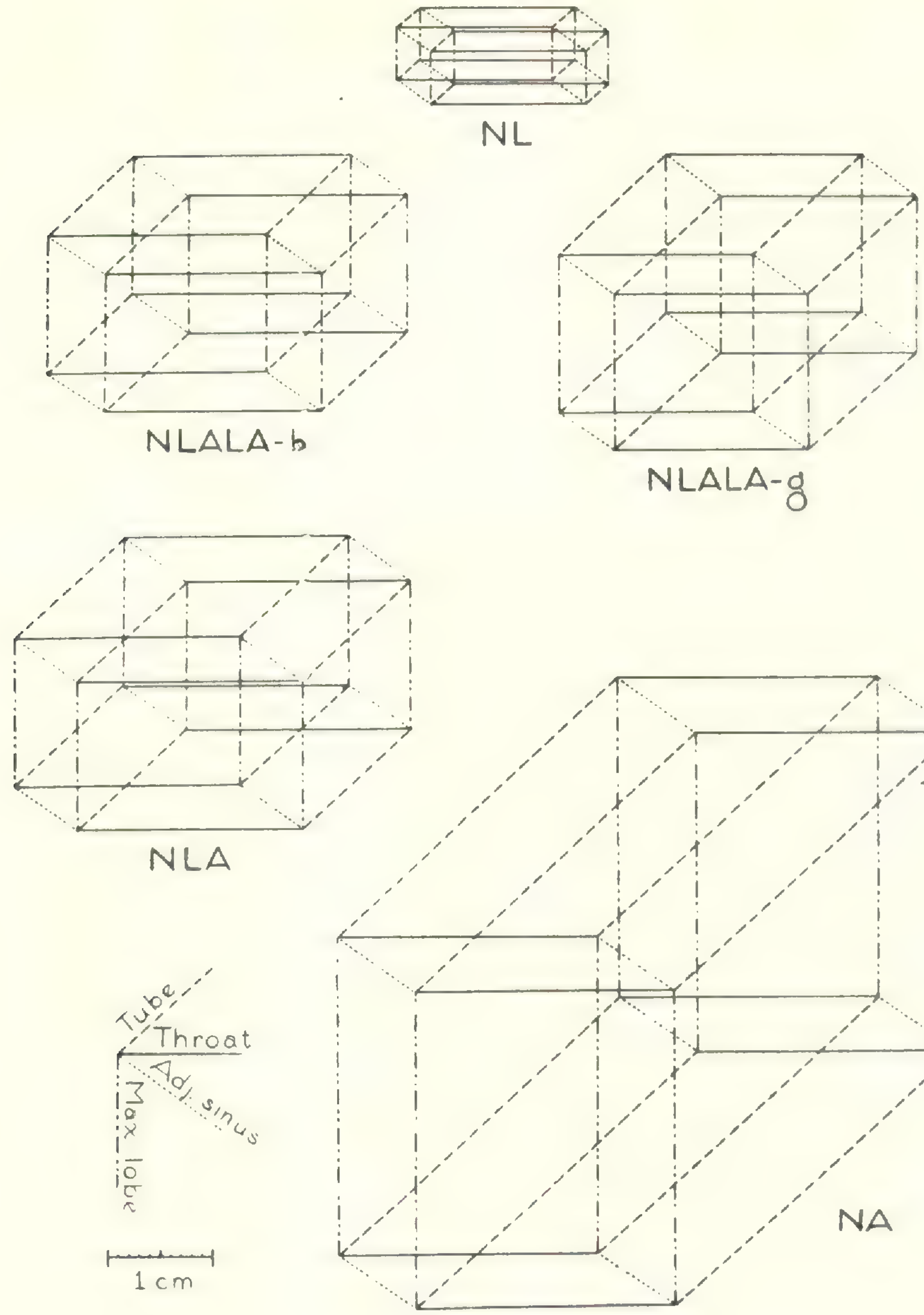

Fig. 2. Fourth-dimensional representation of species crosses in *Nicotiana.*

NL — *Nicotiana Langsdorfii*: throat, 1.5 cm.; maximum lobe, .5 cm.; adjacent sinus, .5 cm.; tube, .4 cm.

NA—*N. alata:* throat, 2.5 cm.; maximum lobe, 3.0 cm.; adjacent sinus, 1.1 cm.; tube, 4.9 cm.

NLA — F-1 generation: throat, 2.15 cm.; maximum lobe, 1.4 cm.; adjacent sinus, .9 cm.; tube, 1.9 cm.

NLALA-b — Family b of F-2 generation: throat, 2.1 cm.; maximum lobe, 1.3 cm.; adjacent sinus, .8 cm.; tube, 1.5 cm.

NLALA-g — Family g of F-2 generation: throat, 1.9 cm.; maximum lobe, 1.5 cm.; adjacent sinus, .8 cm.; tube, 1.9 cm.

RIGHT-ANGLE GRID SYSTEM FOR MAPPING PLANT DISTRIBUTION

ELLEN MARIE KERN

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Unless one has a simple, easily executed method for obtaining an exact sample of plant distribution, the problem of mapping large, heavily populated areas is very difficult. At the suggestion of Dr. Lewis F. Thomas, the usual geographic system of right-angle grids was applied to the distribution of *Taraxacum palustre* var. *vulgare, T. laevigatum,* and their hybrids in a selected area. This method, as shown below, not only gave an exact record of the numbers and distributions of these plants, but it demonstrated certain phenomena, such as the occurrence of well-defined "neighborhoods" of similar hybrids which had not been apparent from mere inspection. By choosing grids of the appropriate dimensions it should be possible to adapt the method to any particular problem.

The area chosen for mapping was a rectangle, 170 x 30 ft., located on the campus of Washington University, between Rebstock Hall and Forsythe Blvd., which was known from reconnaissance observation to possess quite a dense dandelion population showing evidences of apomixis as well as hybridization. In setting up the grid, wooden pegs were used to indicate the corners and intersections. Strings stretched between them formed the lines, the distance between lines being ten feet. The plants along each line were scored for characters previously selected —leaf shape and seed color—and their positions were recorded. Two hundred and fifty sample leaves fell into five leaf-shape groups, A, B, C, D and E (fig. 1).

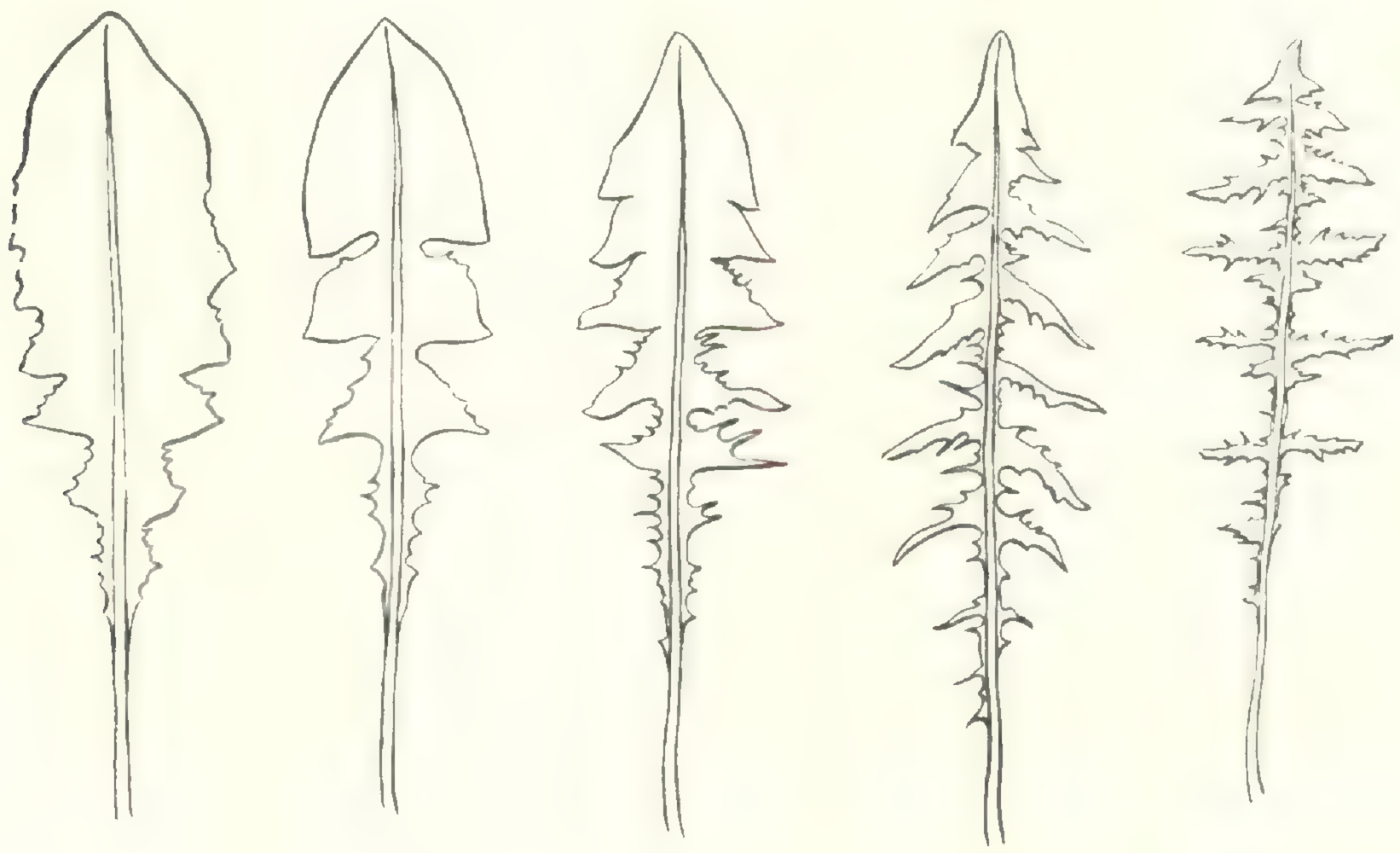

Fig. 1. Types of leaf shape in *Taraxacum*.

The seed types were 1 (gray), 2 (tan), 3 (brown), 4 (pink), and 5 (red). *Taraxacum palustre* var. *vulgare* is characterized by having entire leaves and gray seeds (A-1), while *T. laevigatum* has deeply cut leaves and red seeds (E-5). A map of the grid was drawn to scale. Symbols representing the various features of the dandelions were devised and plotted along each line of the grid (fig. 2 representing a small portion of the map).

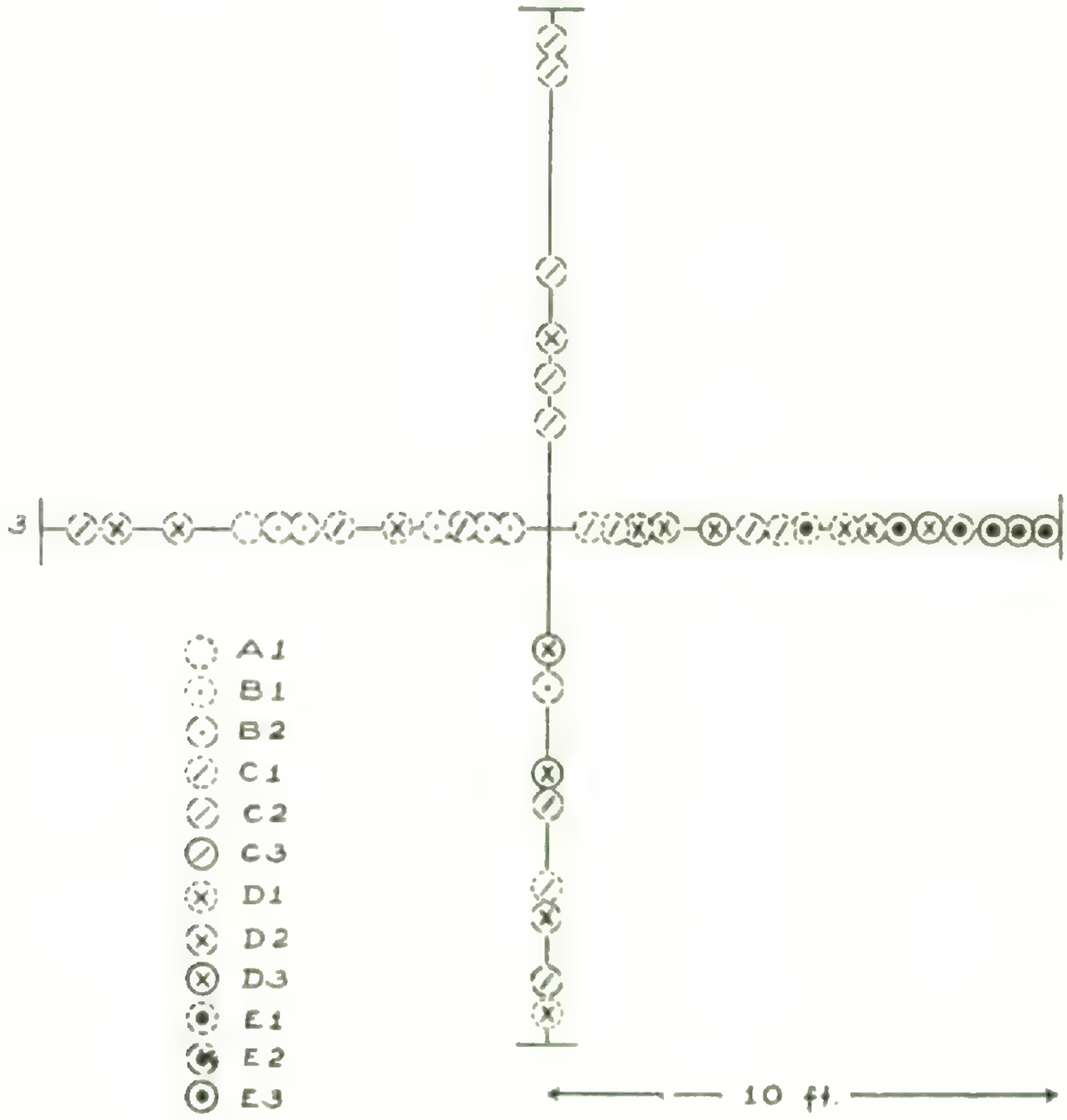

Fig. 2. Individual dandelion plants along Lines II and 3 represented by symbols for leaf shape and seed color.

A simplified map of the total area (fig. 3) indicated certain dandelion "neighborhoods." Plants with dark seeds and deeply cut leaves (similar to *T. laevigatum*) tended to occur in the northern portion of the area; those with gray seeds and entire leaves (similar to *T. palustre* var. *vulgare*) occurred in the southern and eastern part; and the intermediate types with tan seeds and slightly cut leaves were found throughout. The mapping not only made distribution study possible, but also gave data for correlation charts (table I). There is some correlation between leaf shape and seed color, for, although apomixis somewhat clouds the picture, the plants group toward the A-1 (entire leaves, gray seeds) and E-4 (deeply cut, dark) types, rather than the A-4 (entire, dark) and E-1 (deeply cut, gray) types.

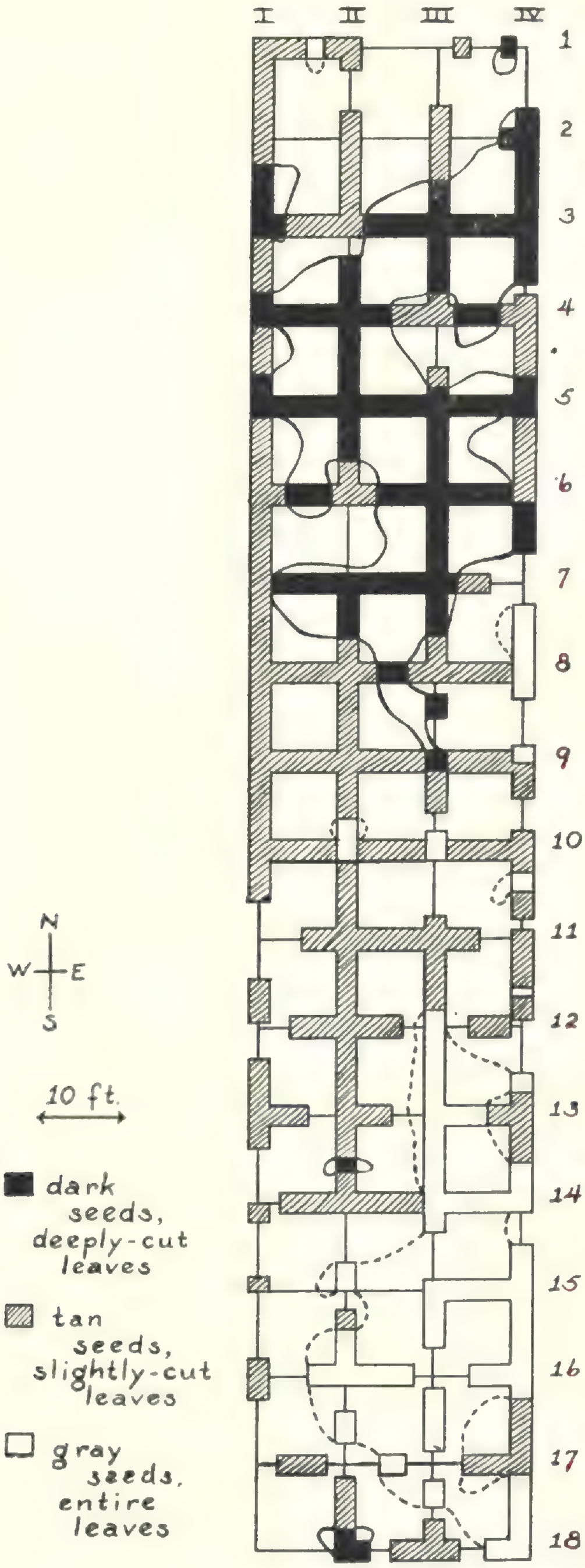

Fig. 3
Grid map of the total area indicating dandelion "neighborhoods."

The right-angle grid technique has the advantage of being adaptable to any type of plant distribution study, merely by increasing or decreasing the distance between lines. For micro-distribution the scale may be brought down to one yard or one foot; for macro-distribution, one mile or ten miles—whatever distance appears suitable. The traverse map, frequently used in plant distribution study, provides just a linear section, whereas the grid supplies the more complete areal view.

TABLE I

CORRELATION OF LEAF SHAPE AND SEED COLOR

Number plants along north-south lines						Number plants along east-west lines					
Seed color	Leaf shape					Seed color	Leaf shape				
	A	B	C	D	E		A	B	C	D	E
1	12	21	12	11	2	1	1	6	8	10	5
2	22	96	64	60	16	2		15	30	25	22
3	9	9	18	18	5	3		1	4	10	12
4			1	1		4					1

In conclusion, I wish to thank Dr. Edgar Anderson for his helpful suggestions, Mr. Richard Holm and Mr. Charles Heiser for their assistance in mapping, and especially Dr. Lewis F. Thomas, who proposed this mapping technique.

A NECTRIA DISEASE OF COFFEE IN WESTERN GUATEMALA[1]

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INTRODUCTION

Although the *Nectria* canker of *Coffea* probably existed in Guatemala more than a decade ago, little importance was attached to it since only an occasional tree died and had to be removed. In 1935 the trunks of all the coffee trees at La Soledad, a large finca near Tumbador, Depto. de San Marcos, were vigorously rubbed with coffee sacking in order to remove the accumulation of algae, mosses, and lichens. That year the crop production was the highest in the history of the finca, but the following year the *Nectria* canker assumed alarming proportions. This fact suggests that the disease had probably existed before and was spread by the rubbings. It is now to be found in scattered spots throughout the finca.

The study of this disease has been in the direction of a definite determination of its specific causative agent and an attempt to cultivate it artificially and to clarify its relationship to the imperfect *Fusarium* stage.[2]

THE PERFECT STAGE

The morphological details of the fruiting bodies were studied from a preserved specimen of the diseased bark. To facilitate the identification of the organism, the specimen was embedded according to the method prescribed by Koneff and Lyons[3]. Sections 7.5, 10, and 15 μ in thickness were made and stained with Heidenhain's iron haematoxylin[4] and safranin.

The organism was discovered to be a species of *Nectria*, a genus which was founded in 1846 by Fries[5] and which was placed in the family Hypocreaceae by Saccardo[6]. It is a genus comprising over 500 species and is divided by Saccardo into ten sections based upon the following characteristics:

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[1] An investigation carried out in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University and submitted as a thesis in partial fulfillment of the requirements for the degree of Master of Science.

[2] The author wishes to express her appreciation to Dr. C. W. Dodge who, in addition to suggesting and directing the problem, supplied the specimens examined throughout the course of this study and the notes of his field observations. The author also wishes to thank Dr. George T. Moore, Director, for the facilities offered by the Missouri Botanical Garden, members of the Botany department faculty, and members of the library staff, for their assistance.

[3] Koneff, A. A., and W. R. Lyons. Rapid embedding with hot low-viscosity nitrocellulose. Stain Technol. **12**:57-59. 1937.

[4] Johansen, D. A. Plant microtechnique. p. 50. 1940.

[5] Fries, E. Summa Vegetabilium Scandinaviae. p. 387. 1846.

[6] Saccardo, P. A. Sylloge fungorum **2**:479-511. 1883.

Issued September 15, 1945.

I. EU-NECTRIA—Perithecia typically smooth, caespitose, and with a stroma.
II. DIALONECTRIA—Perithecia almost separate and smooth.
III. HYPHONECTRIA—Perithecia smooth but seated upon a cottony subiculum.
IV. LEPIDONECTRIA—Perithecia scaly.
V. LASIONECTRIA—Perithecia hairy.
VI. CRYPHONECTRIA—Perithecia somewhat brittle, almost immersed within a stroma.
VII. COSMOSPORA—Spores warted, reddish.
VIII. PHAEONECTRIA[7]—Spores yellow-brown, slightly striate.
IX. ZIMMERMANIA[8]—Ostiole with a toothed crown.
X. LICHENONECTRIA[9]—Parasitic upon lichens.

The species, which falls within the section Dialonectria, may be described as follows:

Nectria Dodgei Heiser, sp. nov.

Perithecia solitaria vel 2–4 aggregata, 140–270 μ lata, 160–250 μ alta, globosa vel ovoidea, laevia, aurantiaca; ostiolum non papillatum; asci ca. 50 x 5 μ; ascosporae hyalinae, uniseptatae, ellipsoideae, 7.6 x 3 μ.

Hab: in cortice *Coffeae arabicae* var. *maragogipes* vivantis in Guatemala.

Type: in Missouri Botanical Garden.

Perithecia solitary or 2–4 aggregate, 140–270 μ broad, 160–250 μ high, globose or ovoid, smooth, orange; perithecial wall pseudoparenchymatous, about 32 μ thick at the apex and 20 μ at the base, composed of 3 to 5 layers of cells; cells at the apex very nearly isodiametric, averaging 15 x 17 μ, cells at the base compressed dorsally, averaging 4 x 13 μ; ostiole not papillate, the canal lined with numerous periphyses; asci about 50 x 5 μ; ascospores hyaline, uniseptate, ellipsoid, not constricted, 7.6 x 3 μ.

Hab: On bark of living *Coffea arabica* var. *maragogipes*, in Guatemala.

SYMPTOMS

The disease which is produced by *Nectria Dodgei* usually appears under good conditions of culture and manifests itself through the following symptoms:

Trunk.—Circular or elliptic cankers occur at the base of the tree and rarely as high as two feet above the soil. Yellow-orange perithecia turning to bright red-brown with age appear on the cankers. The bark becomes blackened, and there is a partial destruction of the tissue which may extend to the cambium. However, below the uninfested areas of the trunk the bark is not blackened, and the lateral roots show no rhizomorphs. Sometimes the canker is near an old machete cut, but not enough trees were examined to know whether this is significant or not.

[7] Op. cit. 11:359. 1895.
[8] Op. cit. 17:787. 1905.
[9] Op. cit. 17:797. 1905.

Roots.—The blackening on the trunk extends down below the soil and along the tap-root to about one foot below the second laterals. Below that the bark appears normal, nearly white. The wood is normal but the cambium is discolored. The outer layers of bark on the upper laterals show cracking and scaling, with embedded black to dark brown rhizomorphs which change to white as the root becomes smaller; the small rootlets are usually unifected. The second laterals, being near the lower limits of the blackened bark, show white rhizomorphs near the trunk and little or no infection near the outer ends. Neither the third whorl of laterals nor the lower portion of the tap-root show any infection.

Leaves.—The leaves above the canker show irregular mottling at first, as in some mosaics, and then become greenish yellow. They are often spotted like *ojo de gallo,* and tend to fall prematurely.

Twigs.—There is frequently some dieback of the twigs as a more or less secondary infection.

Fruit.—The fruit is not directly affected. In fact, the last season before the death of the infected side of the tree, a very heavy crop of fruit is set. However, whether or not it matures depends upon the survival of sufficient leaf surface.

ISOLATION OF THE FUNGUS

Sabouraud's agar inoculated with the *Nectria* ascospores produced only the imperfect *Fusarium* stage. Numerous attempts were subsequently made to induce the production of the perfect stage on various types of media, but only the repeated production of the *Fusarium* resulted.

Since the *Fusarium* species, in general, show great variability in conidial septation and color reactions under different environmental conditions, the study of the cultural characteristics was restricted to cultures grown only on those types of media used by Reinking and Wollenweber[10] in their work on tropical *Fusaria.* By so doing, the results can be compared more easily with those of the above-mentioned authors, who are among the foremost workers on this difficult group of fungi. The microscopical details were studied from smear mounts (lacto phenol acid fuchsin), hanging drop cultures, and celloidin sections.

CULTURAL CHARACTERISTICS

Aerial mycelium white, usually not well developed, smoothly but distinctly warted upon certain media; microconidia[11] straight or allantoid, unicellular, rarely 1-septate, borne in false heads upon simple, occasionally branched conidiophores; macroconidia slightly sickle-shaped, somewhat pedicellate, usually borne in sporodochia; sclerotia and chlamydospores absent. The average measurements of conidia are as follows:

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[10] Reinking, O. A., and H. W. Wollenweber. Philippine Jour. Sci. **32**:103-253. 1927.

[11] This term is here used to designate 1- and 2-celled conidia.

0-septate	2 x 8.5 μ
1-septate	3 x 14 μ
2-septate	3.5 x 24 μ
3-septate	3.8 x 34.8 μ
4-septate	4 x 41 μ
5-septate	4.5 x 43 μ

The septation of the conidia varies considerably with the type of medium used. For example, on oat agar from 90 to 100 per cent of the conidia are microconidia, but on *Alnus* stems, rice, and potatoes, the number of septa ranges from 0 to 5. Even on these last three, the conidia borne on the mycelium are predominantly 0- to 2-septate, whereas those borne in sporodochia are almost exclusively 3- to 5- (mostly 5-) septate. Although the septation varies greatly with the medium, the size of a particular conidial form is essentially constant; that is, unicellular conidia from Substrate A will have approximately the same dimensions as those from Substrate B even though they may be present in greatly different percentages upon the two media.

The following observations were all made of cultures one month old:

Hard potato agar.—Cultures characterized by a medium growth of white to pale pinkish buff[12] woolly mycelium, often with a dull blackish green line in the agar along the line of inoculation, and a raw sienna ring around the base. A cream buff and water green pionnotal mass may be present.

Potato-agar plate, 5 per cent dextrose.—Rather scant, woolly, loosely matted, grayish white aerial mycelium produced over the plate, the agar becoming citrine or vetiver green and sometimes grayish blue green in part.

Oat agar.—Fine cottony pinkish buff mycelium produced at the tip of the slant; the rest of the slant showing little aerial mycelium and being rather powdery in appearance. The agar appears much the same as on the potato-agar plates, and is dark Delft blue near the base.

Rice.—Fine cottony white mycelium produced on top of the medium; mycelium at bottom of the tube light grayish vineaceous. Color of the rice ranges from white through sayal, pecan, and wood brown to liver brown. Heaps of light ochraceous buff pionnotes, which become almost black with age, appear throughout the rice. No benzoic odor noticed.

Potato-tuber plug.—Cultures characterized by white to cinnamon buff felty mycelium that may be honey yellow, forest green, deep grayish olive, and dark Delft blue in patches. Sporodochia may or may not be present.

Alnus stem.—A tuft of fine white mycelium occurs at the point of inoculation, the growth being rather scarce over the remainder of the stem. A number of white and tawny sporodochia are present on the lower portion.

[12] All color terms mentioned in this section are according to: Ridgway, R. Color standards and color nomenclature. 1912.

In addition to the various media mentioned in the preceding paragraphs, a number of others were also inoculated, with the primary object of promoting the growth of the *Nectria* stage. Although in no instance was the *Nectria* produced, some supplementary observations were able to be made concerning the growth of the imperfect stage. Prune agar, coffee-dextrose agar, and potato-dextrose agar (with and without 1 per cent glycerin) all produced much more abundant mycelial growth than any of the media mentioned heretofore. Growth was rather sparse upon Sabouraud's agar and coffee agar, and practically nil upon corn-meal agar. Autoclaved coffee twigs (in a tube having one inch of glass wool covered with 1 per cent glycerin) produced a medium amount of mycelial growth and sporodochia at the base.

Upon prune agar and Sabouraud's agar the mycelium had rather rounded warts, a character not apparent on the other media.

Since several different isolations were made from the diseased coffee trees, cross-inoculations were made upon autoclaved coffee twigs, Sabouraud's, and potato-dextrose agar plus 1 per cent glycerin, in an attempt to discover possible existence of different physiological strains. Here again the results were negative.

COMPARISON WITH OTHER NECTRIAS DESCRIBED ON COFFEA

In order to show how *N. Dodgei* differs from the other species of *Nectria* described as growing on *Coffea*, a key based upon morphological differences, followed by a discussion of physiological differences, is given:[13]

I. Perithecia yellow and clothed with well-developed hairs ..... *N. luteopilosa*
II. Perithecia not clothed with well-developed hairs.
  A. Perithecia reddish brown.
    B. Perithecia caespitose or subcongested; spores sub-fusiform ..... *(N. saccharina)*
    BB. Perithecia densely gregarious; spores ellipsoid ..... *(N. coffeicola)*
  AA. Perithecia yellow, orange, red, or purple.
    B. Spores less than 10 μ long ..... *N. Dodgei*
    BB. Spores more than 10 μ long.
      C. Perithecia over 250 μ in diameter.
        D. Spores ellipsoid or fusoid, 14–15 μ long ..... *(N. anisophila)*
        DD. Spores ovoid, 8–12 μ long ..... *N. tropica*
      CC. Perithecia less than 250 μ in diameter.
        D. Spores 17–20 μ long ..... *(N. coccidophthora)*
        DD. Spores 10–13 μ long.
          E. Spores constricted.
            F. Perithecia sparse to sub-aggregate, red ..... *(N. Behnickiana)*
            FF. Perithecia densely aggregate, yellow ..... *N. fructicola*
          EE. Spores not constricted ..... *N. coffeigena*

Of the ten species listed in the key one has subsequently been transferred to another species of *Nectria*, and four have been transferred to the genus *Hypomyces*. This is readily understandable since the two genera are closely related. Both are in the Hyalodidymae section of the Hypocreaceae, the chief difference being that

[13] *Nectria coccidophthora* has been transferred to *N. coccophila*, and the other species enclosed in parentheses have been transferred to the genus *Hypomyces* by Wollenweber (in Reinking, O. A. and H. W. Wollenweber, Die Fusarien. pp. 34, 132-133, and 159. 1935).

the perithecia of *Hypomyces* are somewhat immersed while those of *Nectria* are more or less superficial.

Probably only four of the species are parasitic on *Coffea*. The remaining six are most likely only saprophytic since they were described from dead leaves, twigs, and fruit, and no indication was given in the original descriptions that they were the cause of any diseases. In addition, none of them has since been reported as the cause of any disease of *Coffea*.

Although *Hypomyces ipomeae (N. coffeicola)*[14] was found on stumps of dying coffee trees, it is doubtful if that fungus was the cause of death because: (1) no mycelium was found in the cambium; (2) the species was also reported on *Melia Azedarach* and dead fruits of *Theobroma Cacao;* and (3) wound inoculations of living trees failed to give positive results. *Nectria luteopilosa* and *N. fructicola*[15] were both found on blackened fruits of *C. liberica* in Java. *Hypomyces ipomeae (N. saccharina)*,[16] *H. ipomeae* var. *major (N. Behnickiana)*,[17] and *N. coccophila (N. coccidophthora)*[18] were found on dead coffee twigs, the last two being reported upon hosts among other genera also.

The chief problem lies, then, in distinguishing *Nectria Dodgei* from *N. tropica*,[19] *H. haematococcus (N. anisophila)*,[20] and *N. coffeigena*,[21] all of which cause diseases of *Coffea*. In addition to the differences in morphological details indicated above, these four organisms may also be distinguished on the basis of their pathological effects upon the host.

The disease caused by *N. coffeigena* is characterized by cankers localized principally at the top of the trunk as contrasted with the basal cankers of *N. Dodgei*. The disease caused by *H. haematococcus (N. anisophila)* was reported from Costa Rica, and although some of the symptoms it produces overlap those produced by *N. Dodgei*, such as blackening of the stem, defoliation, and failure of the fruit to mature, there are several important differences. In *H. haematococcus (N. anisophila)* a large number of rootlets become blackened and partially or totally necrotic and "often the injury extends to the primary and secondary roots."[22] In *N. Dodgei* the root infection is mainly on the first and second laterals and upper portion of the tap-root, while the small rootlets usually remain uninfected. Also the blackening occurs on the basal portion of the trunk below the canker, whereas in *H. haematococcus* it is the young shoots which become blackened.

The other organism which must be distinguished from *N. Dodgei* is *N. tropica*.

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[14] Zimmerman, A. Centralbl. für Bakt. II, 7:101-106. 1901.
[15] *Ibid.* 8:182. 1902.
[16] Berkeley, M. J., and M. A. Curtis. Jour. Linn. Soc. Bot. **10**:378. 1869.
[17] Hennings, P. Hedwigia **44**:172. 1905.
[18] Zimmerman, A. Centralbl. für Bakt. II, **7**:872. 1901.
[19] Toro, R. A. Phytopath. **19**:969-970. 1929.
[20] Picado T., C. Jour. Dept. Agric. Puerto Rico **16**:389-400. 1932.
[21] Pascalet, M. Ann. Cryptogam. Exot. **7**:21-22. 1934.
[22] Picado T., C. *op. cit.*

It was originally described as *N. coccinea* var. *tropica* by Wollenweber[23] from Brazilian coffee collections and later was given specific rank by Toro,[24] who reported it from Colombia. His basis for giving it specific rank was the "contextu radiati-fibrato peritheciorum" character which was entirely wanting in *N. coccinea.* In addition, the spores were much smaller than in *N. coccinea.* This organism causes a disease known under the common name of "llaga" (canker). Toro reports that there are two distinct sets of symptoms. The one is characterized by a dry rot on the upper surface of the roots and the base of the trunk. The bark turns black and partially disintegrates, thus exposing the wood. In the second form, which is said to be rarer, the bark often remains attached and acquires a greenish color. Sometimes it turns soft and gives off a pungent odor. In a few cases the perithecia of *N. tropica* were found on the roots and stumps of coffee trees which had died from this second form. The connection of these two sets of symptoms with the same disease was established upon the fact that in some cases of the second form "the mycelial strands of the first form were also present."[25]

According to Alvarado,[26] who reported the same disease from Guatemala, the canker is more or less extensive over the shoots and roots, and after the death of the tree the fungus forms a single gangrene covering the whole tap-root. From the fact that the fungus gains entrance to the roots through wounds, he concludes that insect wounds and careless cultural practices are predisposing factors of the disease.

Nectria Dodgei, in contrast with *N. tropica,* causes a canker of much more limited extent, and there is no such characteristic gangrene in connection with which the perithecia of *N. tropica* are produced. Furthermore, there is some evidence to indicate that *N. Dodgei* is wind-disseminated, because the infected trees seem to form a linear pattern which corresponds to the direction of the prevailing winds. At the same time, evidence shows that *N. Dodgei* is not spread through the soil, since when clods of soil adhering to an infected stem and upper lateral were carefully scraped off and examined with a hand lens no rhizomorphs were found.

Judging from the three accounts of *N. tropica,* some confusion seems to exist as to the conidial stage, thus raising the question of whether the authors were dealing with the same organism. As Wollenweber[27] described the fungus, the conidia were 5–7-septate. Toro,[28] who reported a *Fusarium* associated with his specimen of *N. tropica* (although the relationship was not proved), could very well have been dealing with the same organism. Alvarado,[29] while apparently dealing with the same organism on the basis of pathological effects upon the host,

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[23] Wollenweber, H. W. Angew. Bot. 8:191. 1926.
[24] Toro, R. A. *op. cit.*
[25] *Ibid.*
[26] Alvarado, J. A. Tratado de caficultura practica. 1:319-320. 1935.
[27] Wollenweber, H. W. *op. cit.*
[28] Toro, R. A. *op. cit.*
[29] Alvarado, J. A. *op. cit.*

states quite definitely that the conidial stage is *Tuberculariа vulgaris*. Since *T. vulgaris* is classed among the Amerosporae, it cannot possibly agree with the conidial stage described by Wollenweber. In spite of the fact that Toro and Alvarado may have been dealing with different organisms, either organism in question may be distinguished from *N. Dodgei* on a pathological basis. Furthermore, Toro's account, which deals with the morphological as well as the physiological aspect, shows that *N. tropica* can be distinguished from *N. Dodgei* on a morphological basis alone, by its much larger perithecium, 380–450 μ, its purple color, and its "contextu radiati-fibrato peritheciorum."[30].

CONTROL MEASURES

In April part of the coffee tree trunks at La Soledad were painted to a height of three feet with lime sulphur or a type of Bordeaux mixture. By the end of May, except in a few cases where normal perithecia were found in the crevices, which evidently had not been completely covered by the brush, the "Bordinette" was effective. It also killed most of the algae, mosses, and lichens expect a species of *Isidium* which remained healthy-looking. Lime sulphur appeared more efficient, however, in the control of both parasite and epiphyte, since it killed everything except *Isidium* and that looked quite sick.

INOCULATION OF COFFEE PLANTS

On March 11, 1944, in the Washington University greenhouse, three 3- to 5-year-old plants of *C. arabica* were inoculated with a spore suspension of a 5-day-old potato-dextrose agar culture of the *Fusarium*. Three plants of *C. excelsa* were also inoculated, and a control was kept of each species. One plant of both species was sprayed in an attempt to learn if the organisms were able to penetrate uninjured tissue; one of each was wound-inoculated near the base of the stem; and the soil in the flower pots containing the remaining two plants was inoculated to learn if the organisms could gain entrance through the roots. After a lapse of one year none of the symptoms characteristic of the disease appeared in any of the plants, but the negative results cannot be accepted as conclusive until the experiment is repeated and revised in such a manner as to eliminate the following possible causes of failure: (1) The only plants available for inoculation were not of the same variety as the ones from which the disease was described and were probably more resistant. (2) Some factor in the environment may have made the test plants more resistant to infection, although every attempt was made to simulate as closely as possible the natural environment of the coffee plants. (3) The prolonged period of culture upon artificial media may very likely have resulted in the loss of virulence of the *Nectria*. (4) The age of the coffee plants tested may also have been a factor in their increased resistance over older plants.

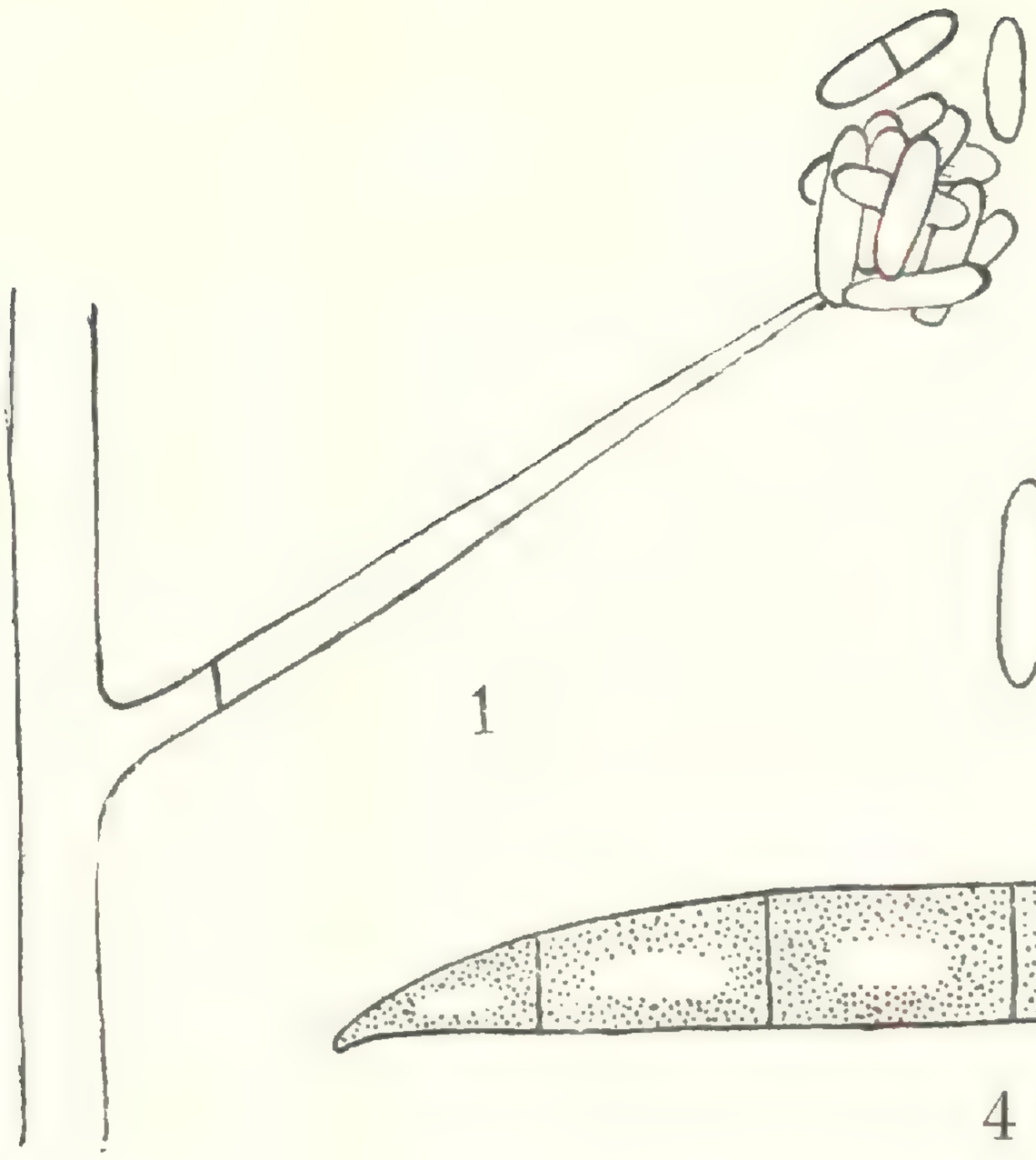
[30] Toro, R. A. Phytopath. **19**:969-970. 1929.

EXPLANATION OF PLATE

PLATE I

Nectria Dodgei

Fig. 1. Microconidia borne in a false head, from a 6-day Sabouraud's agar culture, X 755.
Fig. 2. Two-celled microconidium from a 30-day potato-plug culture, X 1620.
Fig. 3. Unicellular microconidia from a 30-day oat agar culture, X 1320.
Fig. 4. Six-celled macroconidium from a 21-day hard potato-agar culture, X 1500.
Fig. 5. Four-celled macroconidium from a 30-day potato-plug culture, X 1500.
Fig. 6. Warted mycelium from a 7-day prune-agar culture, X 1580.
Fig. 7. Ascospores, X 1500.
Fig. 8. Perithecium, X 175.



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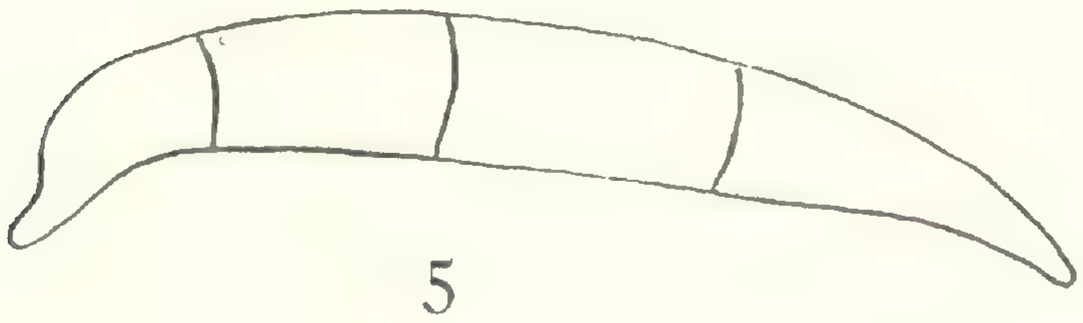

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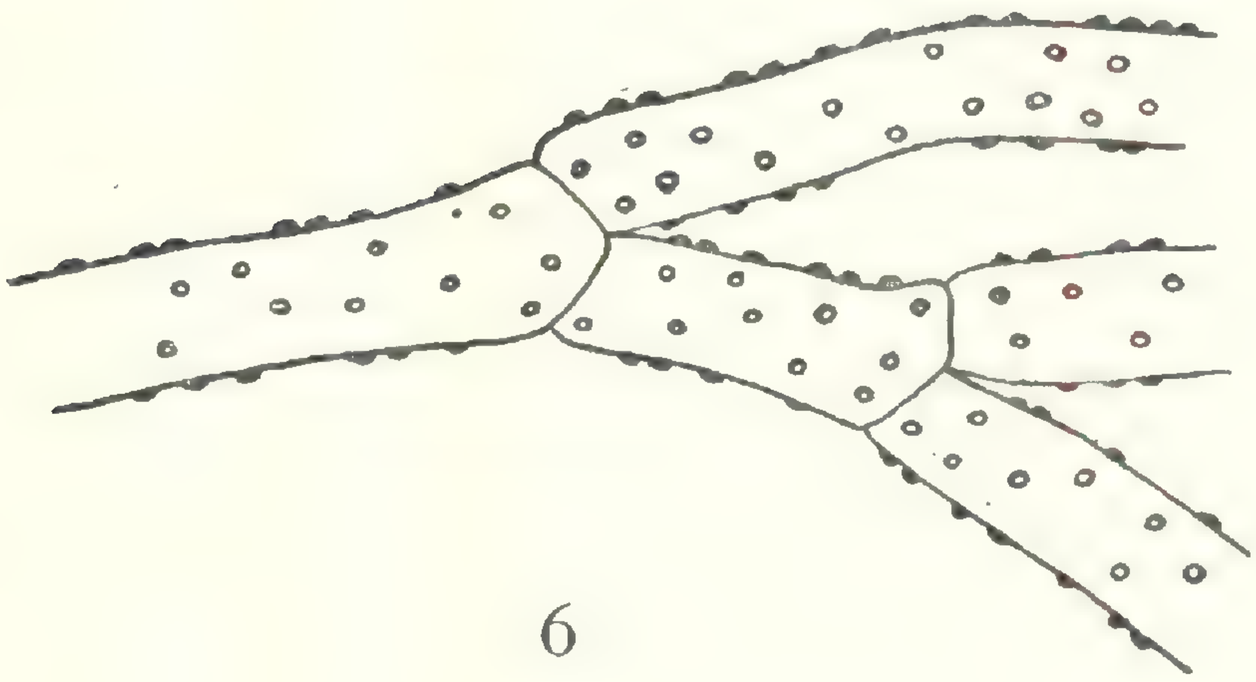

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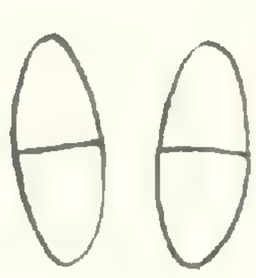

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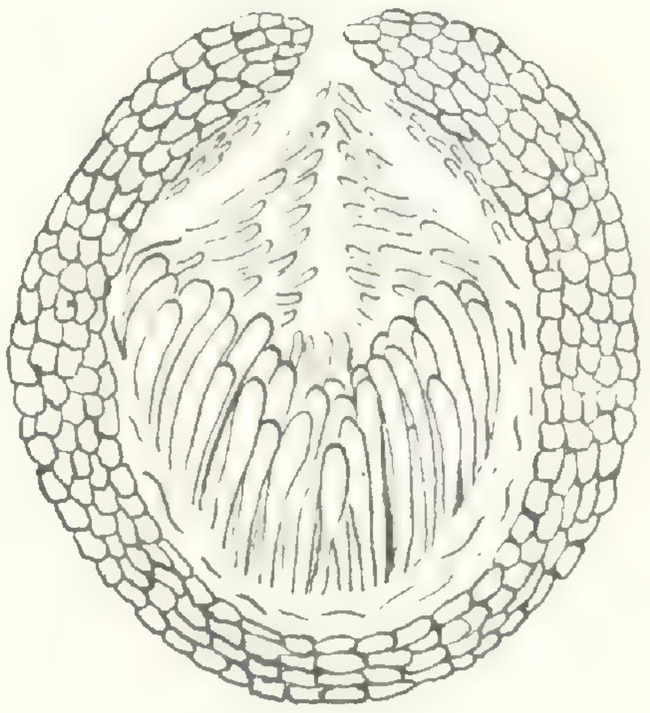

8

A PRELIMINARY SURVEY OF MAIZE IN THE SOUTHWESTERN UNITED STATES[1]

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Maize is uniquely variable; differences from plant to plant, from variety to variety, and from region to region are even greater than in other cultivated plants. Previous papers by Anderson and his collaborators (Anderson and Blanchard, '42; Anderson and Cutler, '42; Kelly and Anderson, '43; Anderson, '43a, b, '44a, b) have discussed the means of cataloguing this variation most effectively. They have recorded it in detail, character by character, and have described a few of the races and sub-races which are already apparent in our collections. The following paper is an attempt to classify the maize of the southwestern United States, particularly the varieties grown by the Indians. While it is more comprehensive than any previous attempt, we consider this a preliminary report since it raises many more questions than it answers, and of these, many should yield to further investigation.

Maize is also unique for purely technical reasons in its excellence for both cytogenetic and archaeological investigation. For cytogenetics it has the initial advantages of many readily available unit characters such as starchy vs. sweet kernels; green leaves vs. purple leaves. For cytological analysis it has the advantage of long, well-differentiated chromosomes. To these original advantages has been added by cooperative research a wealth of detailed technical information unparalleled for any other plant (see Rhoades and McClintock, '35). Archaeologically, maize has the advantage of its large indurated ear which resists decay and which presents almost as many significant characters for racial diagnosis as does the human skull. Therefore, when cytogenetic and archaeological informations are merged we may expect eventually a more complete, detailed, and significant history than is possible for any other cultivated plant or any domesticated animal. Such a synthesis would be useful to geneticists, archaeologists, corn breeders, geographers, ethnologists, and culture historians.

[1] This paper results from the active collaboration of a number of individuals and institutions. The actual funds were supplied by University of California, Guggenheim Foundation, Penrose Fund of the American Philosophical Society, and Missouri Botanical Garden. Laboratory facilities and garden space have been provided by Missouri Botanical Garden, Blandy Experimental Farm of the University of Virginia, Cold Spring Harbor Laboratory of the Carnegie Institution, and California Institution of Technology. Grateful acknowledgment is made to these institutions, as well as to the following individuals: Hugh C. Cutler, E. G. Anderson, A. L. Kroeber, Carl Sauer, E. W. Gifford, Paul C. Mangelsdorf, Barbara McClintock, Volney Jones, O. E. White, Marcus M. Rhoades, Merle T. Jenkins, R. C. Reeves, George F. Will, F. W. Hodges, H. S. Cotton, Emil Haury, and A. F. Whiting.

For a number of reasons the classification of southwestern maize is a relatively simple problem; simple, that is, by comparison with modern commercial maize or the maize of Central America or of South America. Since the beginnings of its agriculture the Southwest has been semi-isolated from other agriculturally developed areas by geographical and climatic factors, and interchange with other areas has been comparatively slight. Within the region itself, maize growing has been strongly localized by climatic conditions; i. e., in contrast to Mexico or Guatemala fields were few and far apart, giving opportunity for the development of well-differentiated local varieties. Compared to most areas, the Southwest is well known ethnologically and archaeologically. Modern and prehistoric maize is already on hand in museum collections[2] in considerable quantity while in practically any other area one must first assemble his own collections. We have artificially simplified the problem even further by leaving out the question of sweet corn. It is grown by a number of southwestern tribes (most particularly by the Hopi), but as Kelly and Anderson have pointed out ('43), the origin of sweet corn is a rather different problem technically from the origin and development of dent and flour corns. The whole question of sweet corn in the Southwest is therefore postponed for further publication.

Unfortunately, collections of modern maize need to be made with extreme care if they are to be of maximum usefulness. Maize is a very sensitive mirror of the people who have been growing it. Collections made from Indians living along concrete highways or in the suburbs of modern American towns will be faithful reflections of the extent to which they have left their old ways. A progressive Papago Indian who owns a small truck and lives on the highway between Sells and Tucson will have seen various kinds of maize in the course of his work and may bring back a good many to try out in his corn field. While most of them may not be well suited to these peculiar desert conditions, if they survive even for one season, the wind may carry their pollen to other plants and a new element will have been introduced into this particular cornfield. Yet the ordinary collector will be satisfied with Papago maize from such a source and will not press on over the long dry road to Sells and then go farther still on side roads into the reservation to find Papago maize which shows no evidence of Yankee contact, none of Spanish contact, and very little contact with other Indians. Yet such maize was characteristic of Papago communities as late as 1943. To some students who have collected maize in the Southwest an Indian was an Indian, and the idea of spending an extra day or an extra week in reaching a seed source of unimpeachable significance seemed a waste of time and effort in a country where travel was difficult. Even those who understood these matters were impeded by the practical necessity of getting back to their base of supplies. The very slight literature on the maize of the southwestern Indians is therefore shot through with information which is only partly true and which would need the joint services of an agronomist and an ethnologist to interpret correctly.

[2] As, for instance, the remarkable collection assembled by Volney Jones and A. F. Whiting at the Museum of Northern Arizona (See Whiting, '39).

In so far as possible our collections were made directly from Indian fields or granaries and from Indians who by ethnological standards were representative members of their groups. The numbers of ears are small (Table II) but the collections are more significant than larger, uncritical ones. Since much of the work was done while both of the authors were traveling from place to place it was seldom possible to measure the entire collection (the bulk of which was divided between Berkeley and St. Louis). Accordingly, for most of the pueblos, the quantitative observations reported below have been confirmed by qualitative observations on a duplicate collection. More recently the junior author has had the opportunity to measure comprehensive collections of twenty-five ears each for eight different varieties. These were distributed as follows: Papago, Tesuque, San Juan, Navajo, Hopi (two varieties), Isleta (two varieties). All these collections confirm and extend the conclusions reached in this paper.

One's first impression of the maize grown in most of the pueblos is that it is extremely variable. This is due to the fact that the eye is quicker to note differences in color than in proportion. Color differences, however, are relatively superficial. In the entire Southwest not more than ten loci in the germ-plasm are responsible for the variation in cob and kernel color, while many more than that are at work in determining cob shape, kernel size, row number, etc. As explained in the first paper in this series (Anderson and Cutler, '42), such characters, though difficult to work with, are therefore superior as criteria for races and sub-races. Color differences, however, have not been ignored but were the object of a special investigation which will be published separately.

One of the characters used in our classification deserves special discussion, partly because it is so important in revealing differences and resemblances between southwestern varieties and partly because its exterior manifestations are somewhat different in the Southwest from what they are in other regions. This character is technically known as denting. In its typical form (pl. 3) it produces a mature kernel which is shrunken at the tip. It is, however, an extremely variable character, and the dent may be either rough or smooth and deep or shallow. When well developed it will affect all the kernels on the ear with the possible exception of a few at the base and the tip. Genetically, we know very little about this character except that it is complex and is apparently affected by a very large number of genes. Our collections of North American maize show that denting reached its strongest development in the region around Mexico City and radiates from there in all directions. In the Southwest it is present but in a very diluted state. Many of the ears which we have scored as dented (pl. 3) would not be referred to as dent corn in the American corn belt, but upon careful examination several of the kernels show a slight dent in the surface of the kernel. Examination of granaries and maize fields have shown us that when such ears are found, invariably sister plants occur with kernels much more strongly dented. Archaeologically, denting is important because it indicates some kind of connection with the Mexican Plateau and because it is one of the consistent differences between

Pima-Papago and Basketmaker maize. When and by what route it entered the Southwest is a matter of great archaeological importance. Collections of prehistoric maize in the Southwest should be carefully examined for any indications of dented kernels. They are illustrated, though without comment, in Nussbaum and Judd's collections from Cottonwood Cañon in southeastern Utah, and the remarkable ears collected by Scoggin in northwestern Colorado (which were submitted to us for study) are as strongly dented as Mexican varieties.

Maize in the Southwest stems from at least four different sources. (1) The race grown by the prehistoric "Basketmakers," the first agriculturists of whom we have any record in the area, was relatively uniform. It was slender-cobbed, tesselate-seeded (like tiles in a pavement, see pl. 2), compressed at the butt, and with strong husk striations. .Unfortunately, not enough plant material has been saved in archaeological studies to indicate the plant type associated with this type of ear. (2) Similar (though apparently not identical) varieties are still being grown by the Pima, the Papago, the Yuma and related tribes. They too are slender-cobbed, tesselate-seeded, and striated. The plants of these modern varieties are slender-stalked and narrow-leaved and have tillers which are sub-equal to the main stalk.

The maize of the Pueblo Indians shows strong influence from at least two other strains. It would be out of place in this preliminary discussion to go into the complex problem of just where and when the modifications took place. The old types were not completely replaced, for most of the Indian maize of the Southwest has a very strong resemblance to Basketmaker corn (as will be demonstrated below). Regardless of source, the two later introductions brought in characters which are identified with two other regions. (3) Condensed tassels (Anderson, '44b), denting of the kernel, weak leaves, a strongly tapered ear, and high row number are characteristic of the maize of the Mexican plateau. (4) Present as an admixture in Pueblo corn is a complex of characters which in its most extreme form is apparently limited to Guatemala. It is, however, markedly present in the flint and flour corns of the eastern United States though absent from most of Mexico. It is characterized by strong, arching leaves, coarse stalks, a large indurated shank below the ear, and an ear whose butt end is perceptibly larger than the rest of the kernel-bearing portion. The kernels are wide, often wider than they are long, and are arranged in long, straight, regular rows, aside from the base of the cob where the rowing is usually irregular. Throughout the remainder of this paper these last two complexes will be referred to as "Mexican" and "Eastern." These names are chosen for convenience and the two complexes may have come into the region separately or together. It is even possible that the one we are calling "Mexican" may have entered from the Northern Periphery.

If we postpone the historical interpretation of these complexes and merely use them as cataloguing devices, the results are suggestive. Figure 1 shows the data on a Mexican character (denting) and an Eastern character (wide kernel) set out in the form of a scatter diagram. A similar, but by no means identical, diagram

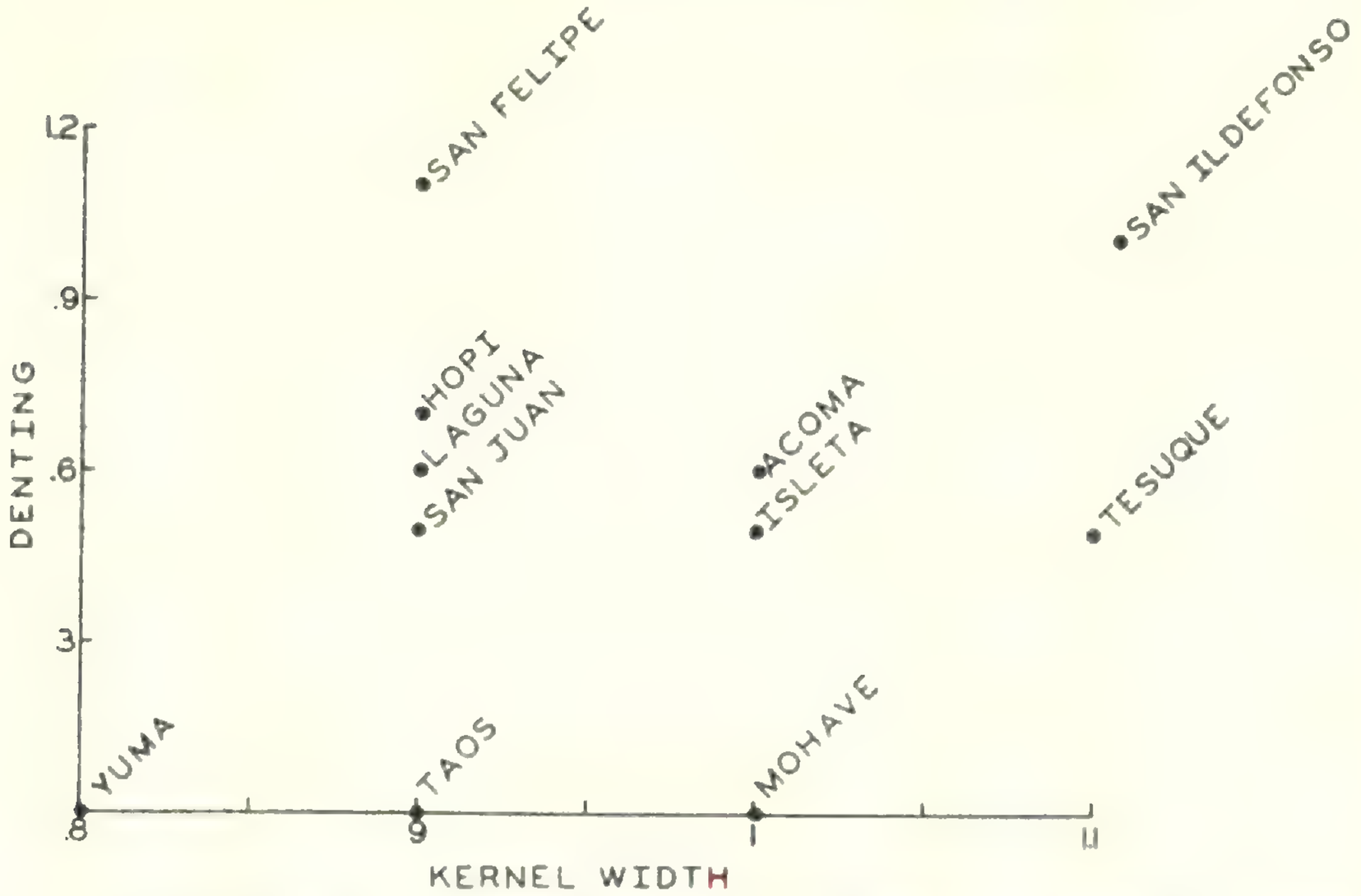


Fig. 1. Scatter diagram showing the relationship between the varieties of maize grown by various tribes in the Southwest. Each dot is an average of all the collections from the tribe.

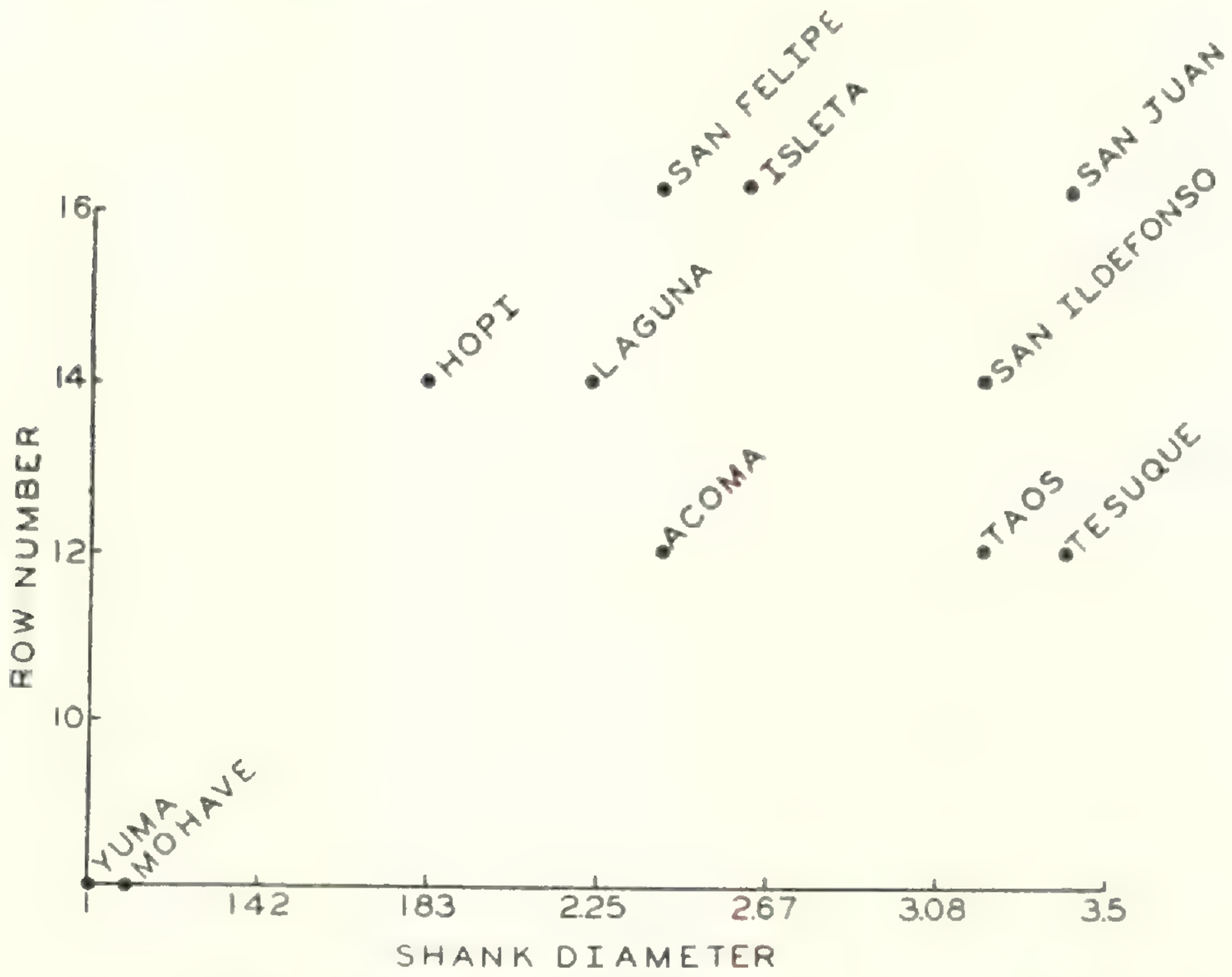


Fig. 2. The same collections as diagrammed in fig. 1, but using two different characters. In both figures a "Mexican" character has been used on the vertical axis, and an "Eastern" character on the horizontal axis.

(fig. 2) is produced if we choose two other characters, taking row number from the Mexican complex and shank diameter from the Eastern. In figs. 1 and 2 all the ears of maize from each group of Indians have been averaged and the average

values were used in making the diagrams.

The fact that figs. 1 and 2, though based on very different criteria, produce such similar diagrams of relationship, is evidence for the validity of the complexes described above. It is evidence that the combinations of characters which tend to be seen together are not wholly fortuitous and that their resemblance to races of maize in Mexico and in eastern North America probably has an historical basis. In terms of genetics it probably means that row number and denting are both multiple factor characters and that, though there has been a great deal of crossing of different types of corn in the Southwest, high row number and denting went into the mixture together (we now have archaeological evidence for this fact) and still tend to stay together on the average.

The existence of these loosely linked complexes of characters in southwestern maize allows us to extend a technique originally developed for dealing with hybrid populations (see Anderson and Turrill ('38) for details). In practice this was done step by step. When the two diagrams (figs. 1 and 2) were averaged mathematically it was found that while either one produced a fairly natural classification of the tribes and one that was in harmony with what was known about the history of their agricultural relationships, the combination of the two was superior to either alone. Row number and denting were used together as an index of the Mexican complex, and shank diameter and kernel width as an index of the "Eastern" complex. Additional characters were added to trial indices, one at a time, until at length the ten most objective and easily scored criteria were being combined in each diagram, each of the characters being given equal weight in constructing the indices.

All of the qualitative characters are put in three grades, "present", "intermediate" or "absent," which are assigned numerical values of 2, 1, and 0, respectively. Each of the quantitative characters is divided into three classes, likewise scored from 0 to 2. The actual scoring for all ten characters is as follows:

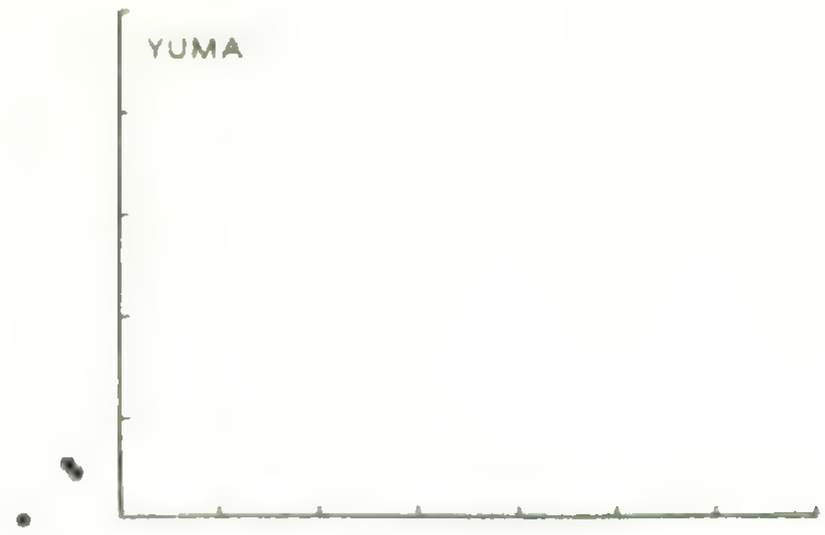
TABLE I

		0	1	2
"Mexican"	Ear taper	None	Slight	Well-marked
	High row number	8–12	14	16 or more
	Denting	None	Slight or variable	Uniform or deep
"Eastern"	Enlarged butt	None	Slight	Strongly developed
	Straight rows, uniform kernels	None	Moderate	Kernels very uniform
	Wide kernels	to .9 cm.	1.0 cm.	1.1 cm. or more
	Wide shank diameter	to .9 cm.	2.0–2.6 cm.	2.7 cm. or more
Non-Mexican Non-Eastern	Mid-ear diameter	to 4 cm.	4.1–4.3 cm.	4.4 cm. or more
	Husk striation	None	Slight or variable	Strongly developed
	Basal compression of ear	None		Strongly developed

The first three of these characters (ear taper, row number, and denting) are characteristic of the Mexican complex. They are all added and their sum is plotted on the y axis. The next four (enlarged base of ear, regular kernels, width of row, shank diameter) belong to the Eastern complex. Their sum is plotted on the x axis. We come now to a cluster of traits which do not characterize either the Mexican or Eastern complexes but which are found in both Basketmaker and Pima-Papago maize: a narrow cob, longitudinal striations across the face of the kernels, and basal compression of the ear. Depending upon the way these traits were defined and scored they might either be added to each of the indices or subtracted from each. If husk striations, for instance, were scored for their absence then the resulting score would be *added* to both the Eastern and Mexican indices. If scored for their presence then the resulting values would be subtracted for both indices. We found that for rapid scoring of large collections errors were apt to creep in if some characters were being scored in a negative and others in a positive direction. Accordingly, in taking the original data we scored all the characters positively. This complicated the mathematical treatment a little, but added to the accuracy of the data. Mid-ear width is a character by which both the Eastern and Mexican complexes differ from Basketmaker or Pima-Papago corn. Its score is accordingly divided by half and the halves are added to both the x and y axis. Husk striations on the kernel and compression of ear at the base are characteristic of Basketmaker and Pima-Papago corn and of neither the Eastern nor the Mexican complex. Their scores are accordingly added, the sum is halved and the result subtracted from both the x axis and the y axis.

All of this sounds rather complicated. In practice the calculations are really very simple. Let us take an actual instance. The ear known in our collections as CA. #240 was collected at the San Ildefonso pueblo. It is intermediately tapered, has 14 rows of kernels, and is undented. These three characters therefore contribute 1, 1, and 0, respectively to the index of the Mexican complex. The base of the ear is not perceptibly enlarged, the kernels are regular in shape and arrangement, they are 10 cm. wide, and the diameter of the shank just below the ear is 3.1 cm. By the score values as tabulated above, these four characters therefore contribute 0, 2, 1, and 2 to the Eastern Index. Adding the above individual scores gives sub-totals of 2 for the Mexican Index and 5 for the Eastern. The mid-ear width is 4.3 cm. which is in the intermediate range and therefore scores 1, giving us ½ to be added to each axis. The ear is not compressed at the base and has slight husk striations on the kernels. Its score for these two characters is therefore 1 plus 0. Dividing this by 2 gives ½, which when subtracted from both totals cancels the previous ½ and gives us total scores of Mexican 2, Eastern 5. This ear therefore appears as a dot in fig. 15, two units up from the 0 point and 5 to the right.

The scale for the Mexican complex runs from a minus 2 to a plus 7 and the scale for the Eastern from a minus 2 to a plus 8. Theoretically, an idealized Eastern ear should score: y, 0; x, 9. Several ears of Sacred Flour Corn from the


YUMA

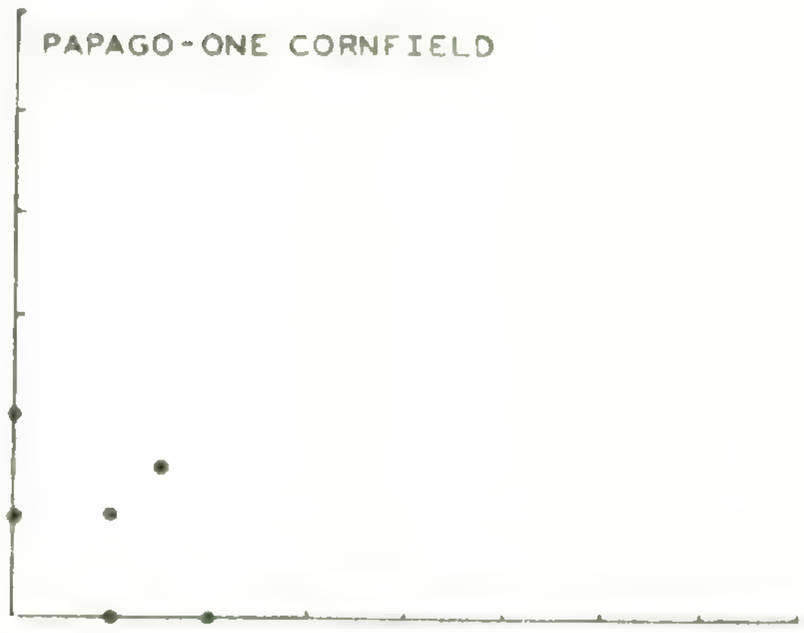
3

MOHAVE

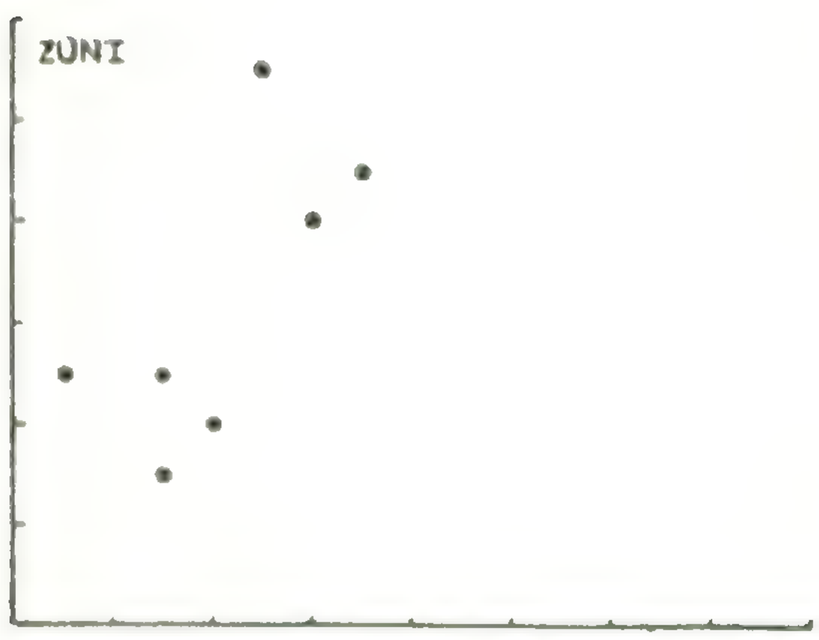
4

HAVASUPAI

5


PAPAGO-ONE CORNFIELD

6


ZUNI

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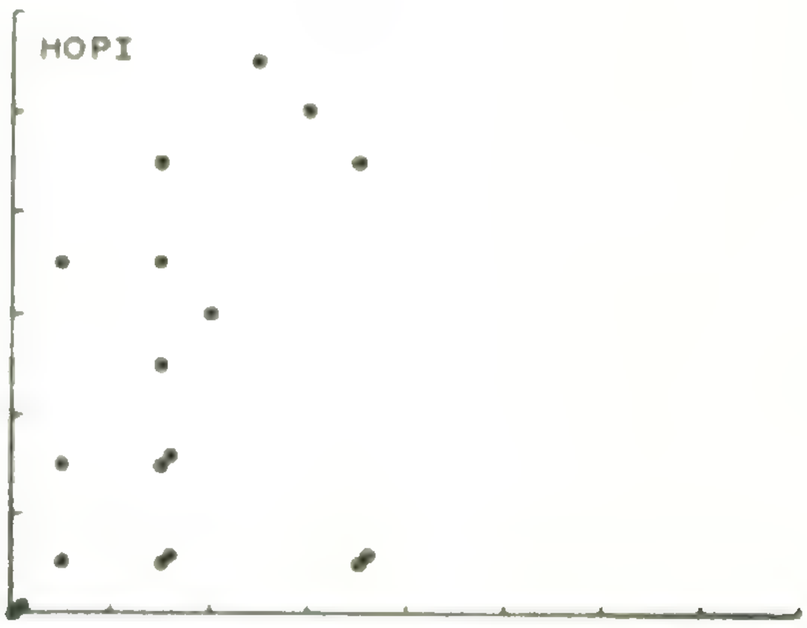
HOPI

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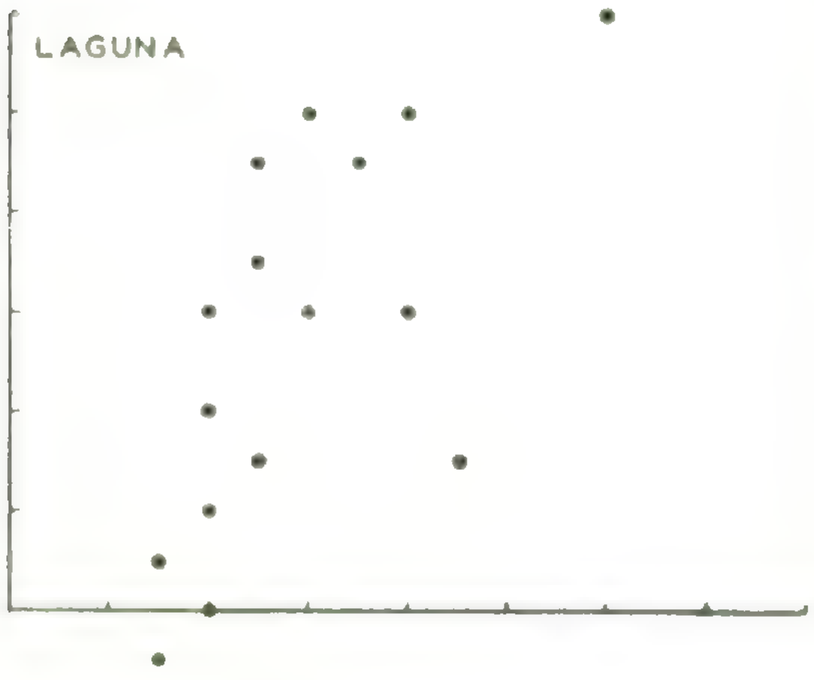
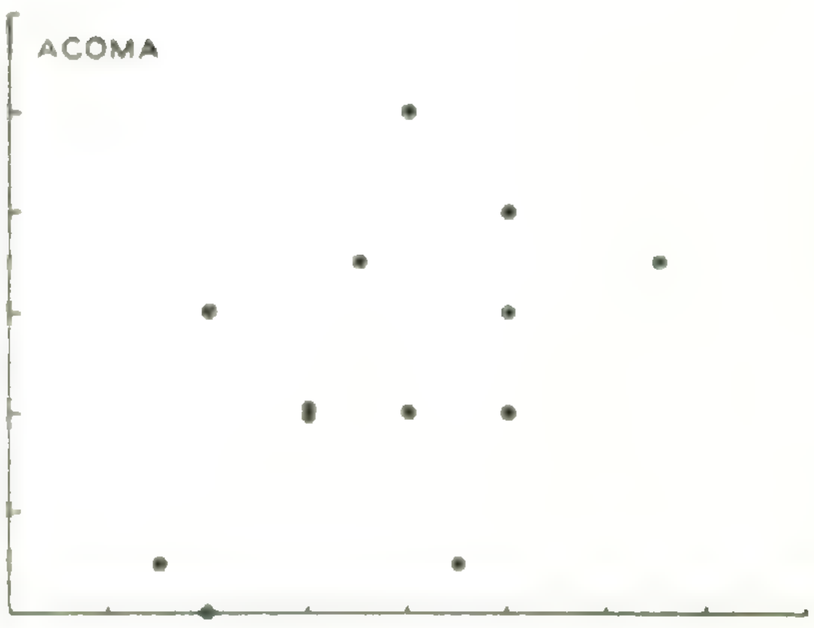

LAGUNA

9


ACOMA

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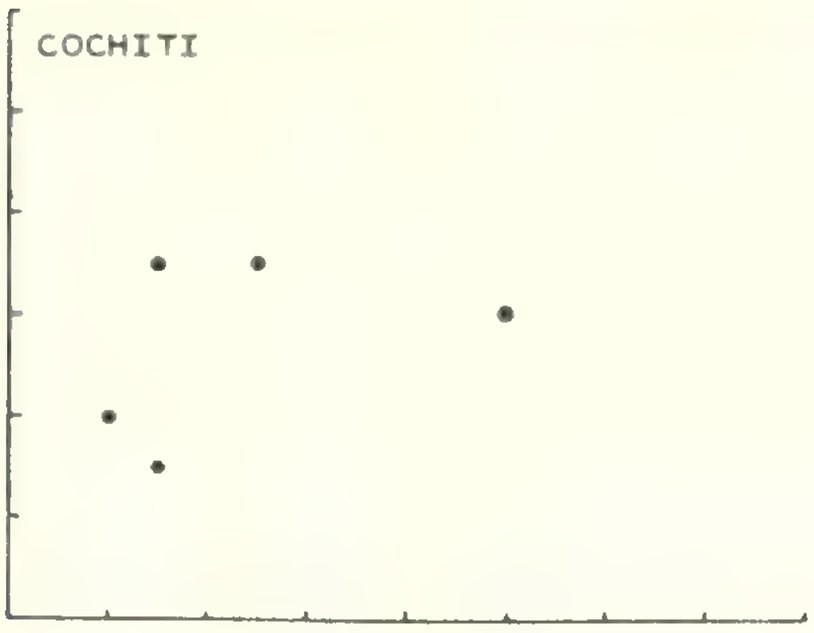

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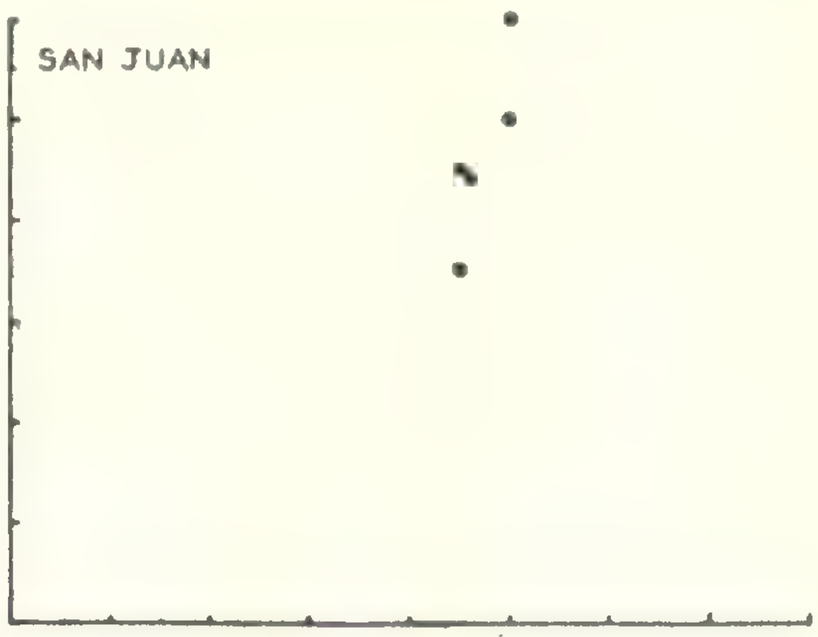

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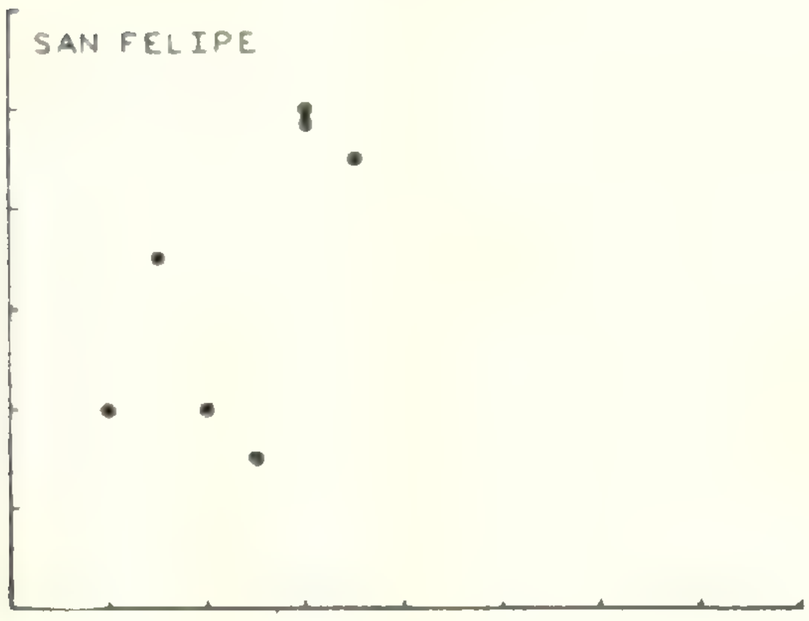

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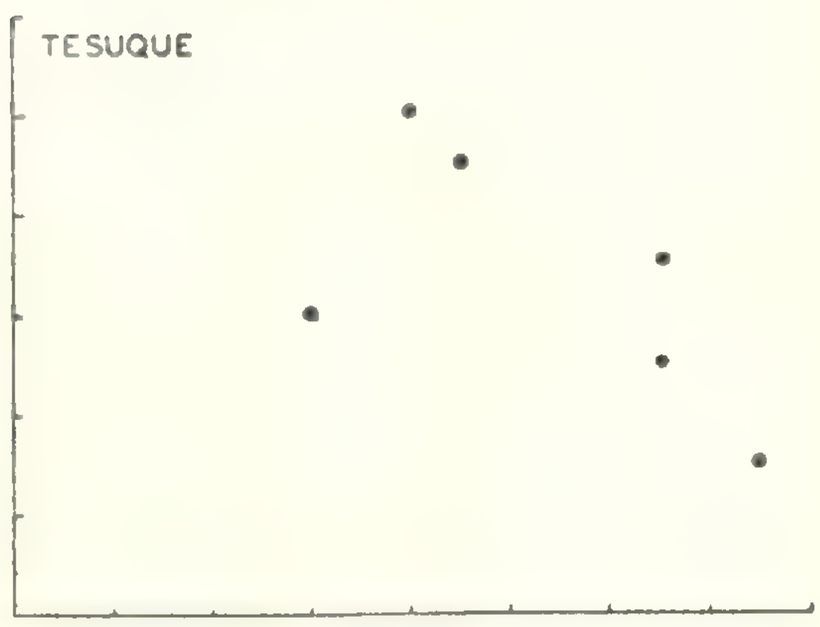

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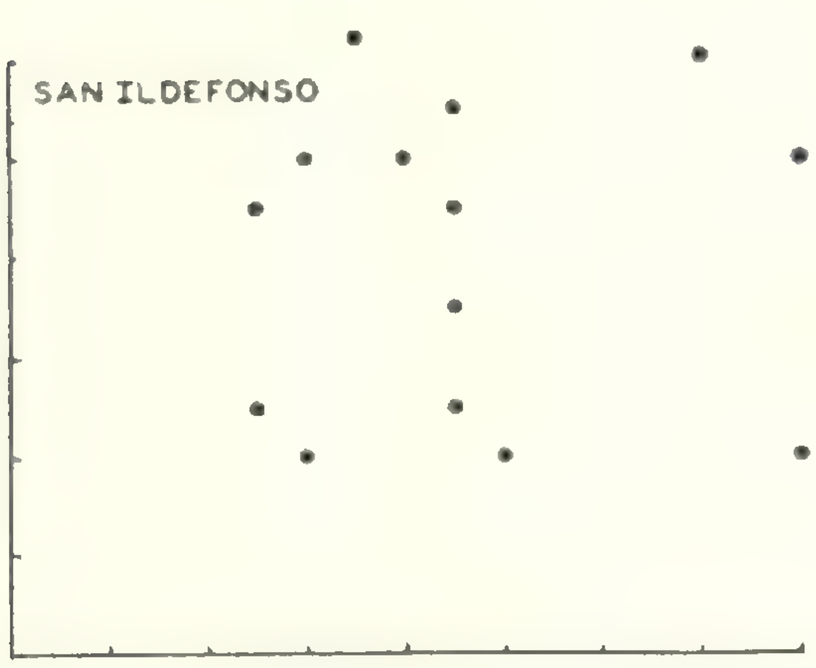

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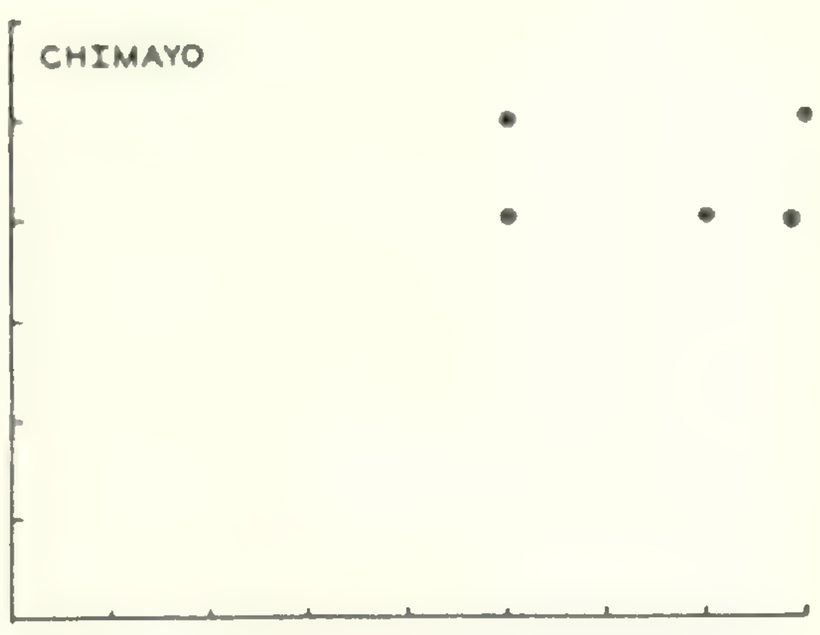

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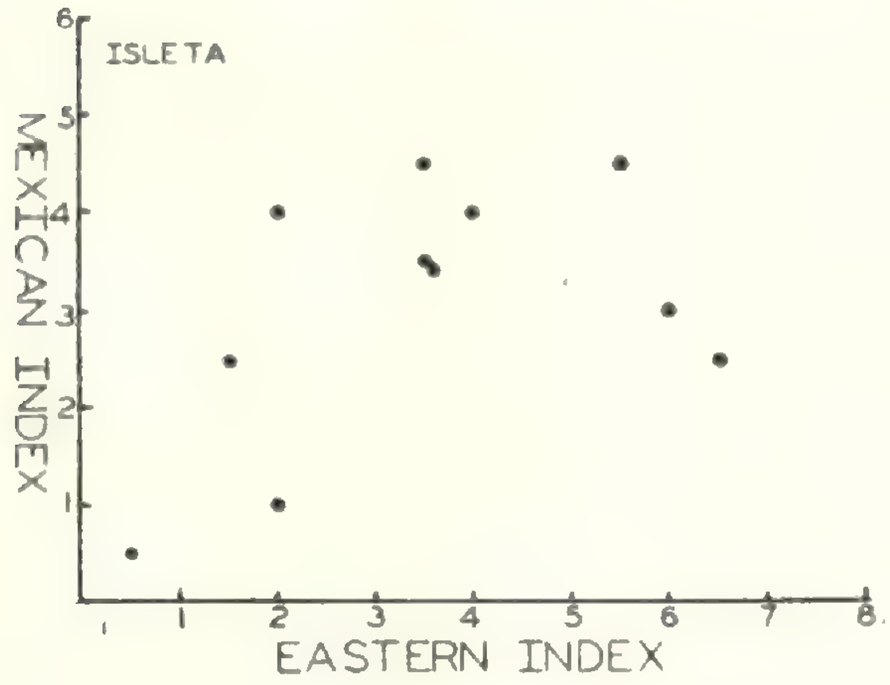

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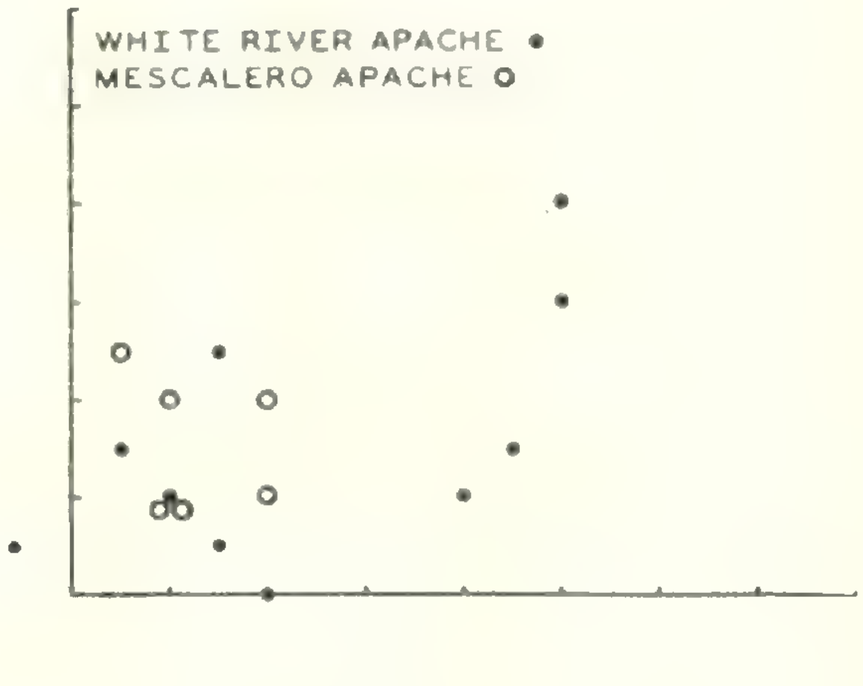


18

Figs. 3–18. Scatter diagrams showing individual ears of maize measured according to a "Mexican Index" on the vertical scale and an "Eastern Index" on the horizontal scale. Further explanation in text.

Six Nations of New York state all were found to score: y, 0; x, 8. One ear of a primitive pointed dent maize from Michoacán, Mexico, scored: y, 7; x, 0. Much of the corn from the neighborhood of Mexico City scores: y, 6; x, 2. The material in our collections from the Pima, the Papago, and prehistoric Basketmaker maize scores either —2, —2 or somewhere between there and 0, 0.

The two indices plotted at right angles to each other give us, therefore, a comparison grid 9 units high and 11 units long. Its upper left-hand corner represents a sharply tapering, highly dented Mexican corn and its lower right-hand corner the big "crescent-seeded" types of eastern North America. The upper right-hand corner should denote corn in which both the Mexican and Eastern complexes are highly developed. It would therefore be a large-eared, large-shanked, wide-leaved corn, highly dented with many rows, and a markedly tapering ear which bulges at the base. Such a type of corn is fairly common in the more highly developed maize-growing regions in Mexico. In the Southwest it spread to such old Spanish-American communities as those at Santa Fe and Chimayo.

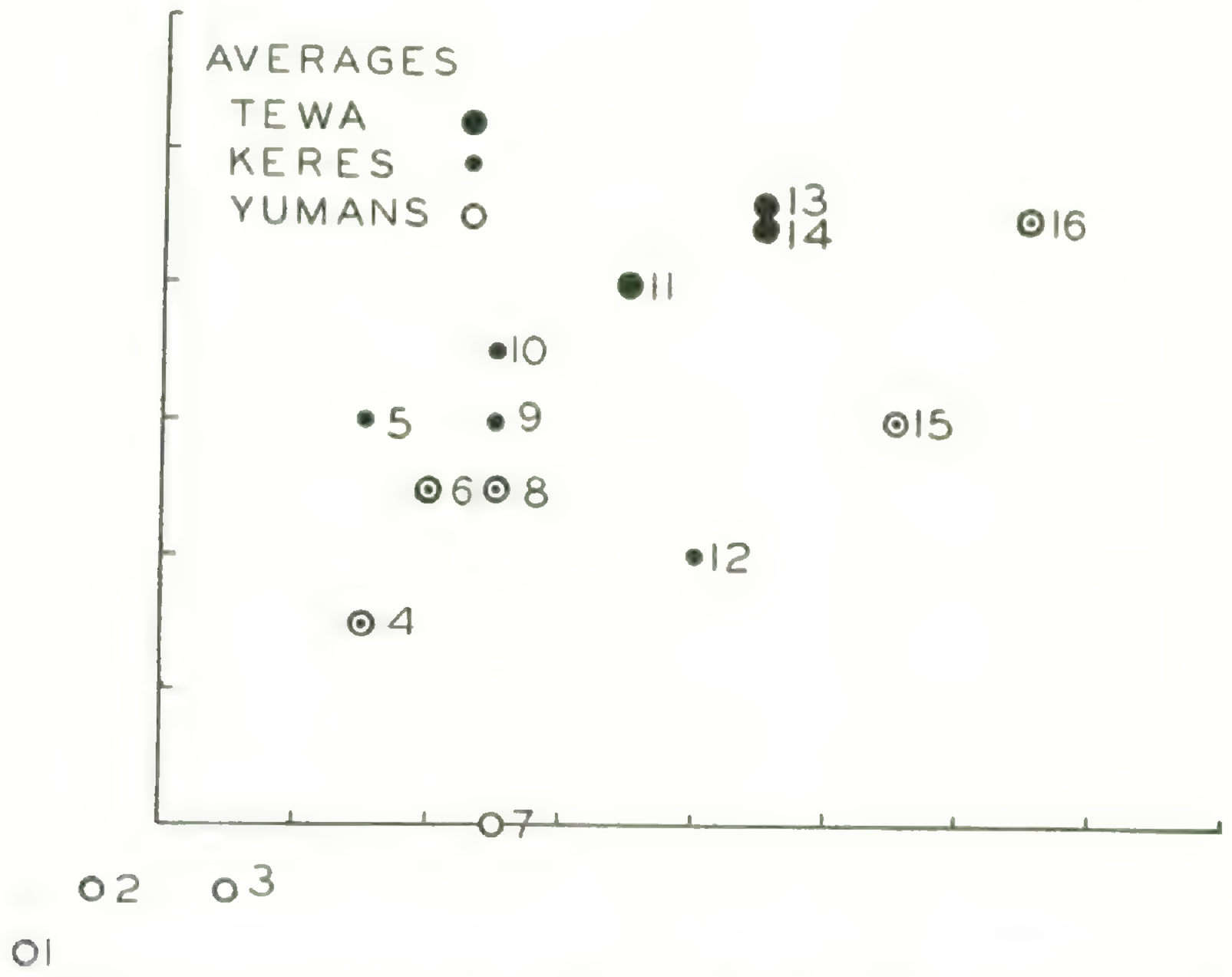


Fig. 19. Data of figs. 3–18 averaged by tribes. Language groups represented in part. Each symbol represents the average of all the measured collections from one tribe as in figs. 1 and 2. Dot-in-circle symbol is for various tribes not falling in one of the three designated language groups. 1 signifies Papago; 2, Yuma; 3, Mohave; 4, Hopi; 5, Cochiti; 6, Zuñi; 7, Havasupai; 8, Navaho; 9, Laguna; 10, San Felipe; 11, Isleta; 12, Acoma; 13, San Juan; 14, San Ildefonso; 15, Tesuque; 16, Chimayo.

We can use the grid in two ways, either to show the morphology of single ears with relation to these four extreme types or to compare the theoretical averages of particular collections. Our data from the Southwest are presented, tribe by tribe, in figs. 3–18. In fig. 19 the averages of each tribe are given for

comparison with each other. In fig. 20 the measured ears in our original collection are diagrammed, using the same grid as was used in figs. 3–18. When the entire lot is inspected in fig. 20, it is apparent that the maize of the desert-dwelling Indians is fairly well set off from that of the Pueblos. The Puebloan maize can be more or less arbitrarily divided into an intermediate type, and into Puebloan, which varies from strongly Eastern to strongly Mexican. In Table II the entire collection is presented in a tabular summary according to this classification.

When the above classification had been completed, instead of drawing dots on paper, a large table top was marked off with the appropriate squares and the actual ears were laid out on the enlarged grid. After this was done it was evident that the classification was quite a natural one (Anderson and Cutler, '42), since it put similar qualities together in addition to those which had been used in making the indices. This was particularly noticeable for color; the whites were strongest around the 0, 0 point and the red pericarp colors (P) were concentrated in the upper right-hand corner of the figure.

In short, an analysis of the color differences brings out the same points as the analysis of the shape and size differences presented above. Color is such a technically complex problem genetically that its analysis has been undertaken jointly with Dr. E. G. Anderson and the results will be published separately. The chief points brought out by this analysis are as follows: In so far as their color genes are concerned, the maize varieties of the Southwest are of two extreme types with numerous intermediates. One of these extreme types prevails among the Papago

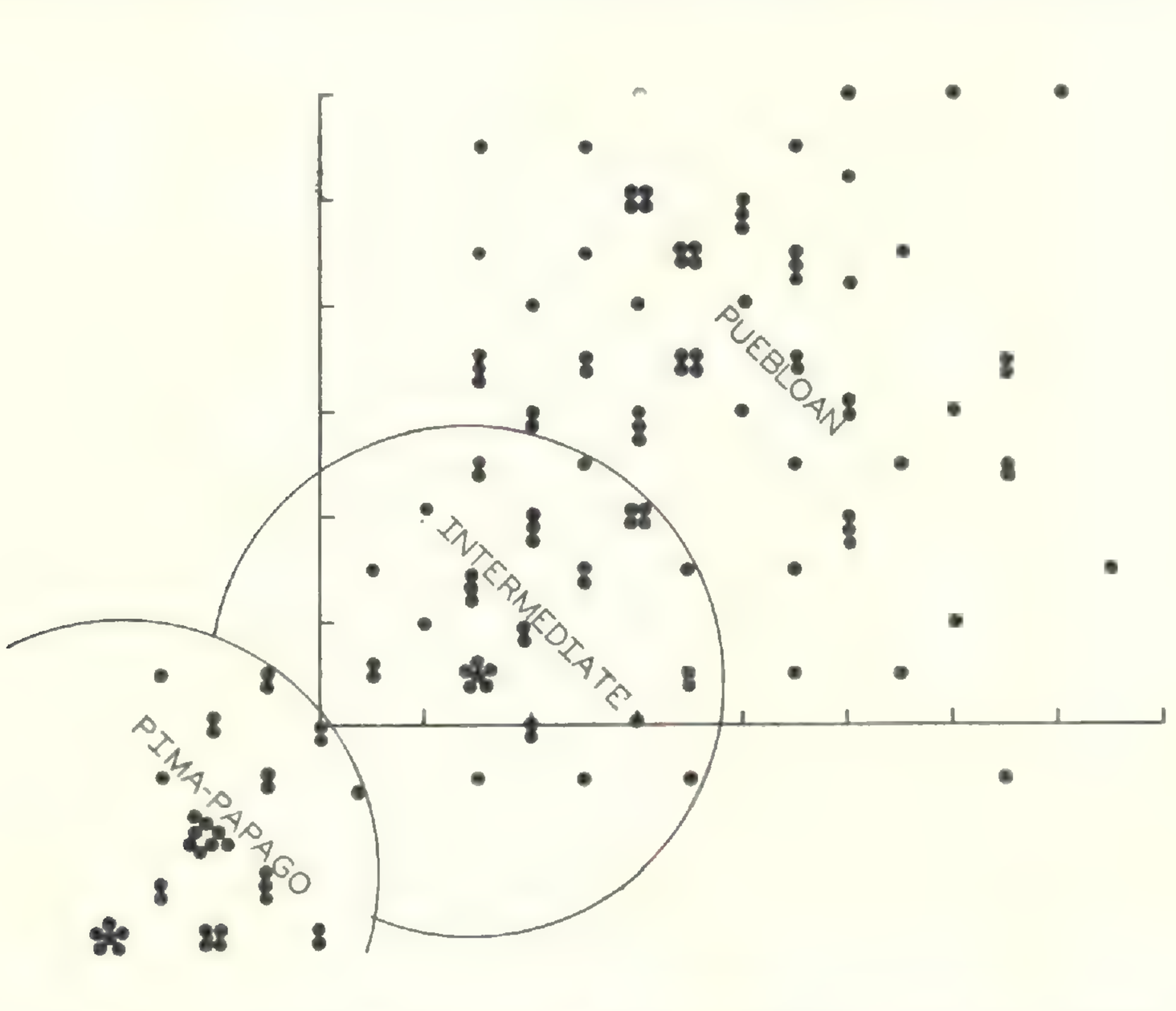


Fig. 20. Scatter diagram showing all the measured ears from the Southwest, using the same two indices as in figs. 3–19. Each dot represents a single ear.

and related tribes. It is a gene combination (ccrr) apparently common in west Mexico and in South America. It differs only slightly (though significantly) from that of Basketmaker maize. The other extreme type is characteristic of the eastern Pueblos while the maize of the western Pueblos consists of various combinations of these two extremes.

TABLE II

MEDIAN VALUES

	Number of ears measured	*Prevailing sub-race or races of maize*	*Kernel diameter in mm.*	*Number of rows*	*Mid-cob diam. in mm.*	*Shank diam. in mm.*
Desert Indians						
Papago	62	Pima-Papago	9	12	36	14
Yuma	7	Pima-Papago	8	10	32	10
Mohave	5	Pima-Papago	10	10	37	11
Hopi & Neighbors						
Havasupai	10	Pima-Papago & Intermediate	11	12	41	19
Hopi	19	Intermediate & Puebloan	9	14	38	18
Zuñi	7	Intermediate & Puebloan	9	16	40	23
Keres-speaking Pueblos						
San Felipe	7	Intermediate & Puebloan	9	16	41	24
Acoma	13	Intermediate & Puebloan	10	12	42	24
Laguna	16	Intermediate & Puebloan	9	14	43	22
Cochiti	5	Intermediate & Puebloan	9	16	44	24
Tewan Pueblos						
San Juan	5	Puebloan	9	16	45	34
Taos	6	Puebloan	9	12	42	32
Tesuque	6	Puebloan	11	12	43	34
San Ildefonso	14	Puebloan	10	14	44	32
Isleta	10	Puebloan	10	16	45	26
Nambe	3	Intermediate & Puebloan	10	14	38	30
Jemez	3	Puebloan	9	16	46	28
Intrusives						
Mescalero Apache	6	Intermediate	8	14	38	16
White River Apache	10	Intermediate & Puebloan	10	12	42	18
Navajo	26	Intermediate & Puebloan	10	14	41	22
Chimayo Sp. Amer.	4	Spanish	12	14	51	22
Santa Fe Sp. Amer.	3	Spanish	12	14	52	24

A number of facts are apparent from figs. 3 to 20:

1. The maize of the desert Indians (Yuma, Mohave, Cocopa, Pima, Papago) is comparatively uniform and essentially similar. It is not very different from the maize of the widely distributed prehistoric Basket-makers.

2. The maize of the pueblo-dwelling Indians is much more variable both as to the varieties grown by a single tribe and as to differences between tribes. In general, the maize of the eastern Pueblo people is much more Eastern-like than that of the western Pueblos.

3. Of all the Pueblos, the maize of the Hopi shows the least Eastern influence. Some of it is almost identical with Basketmaker maize. As a whole, it is rather like such corn which has been strongly influenced by the Mexican complex.

4. Zuñi maize is much like Hopi, but in our admittedly incomplete collections it lacks the Basketmaker-like varieties.

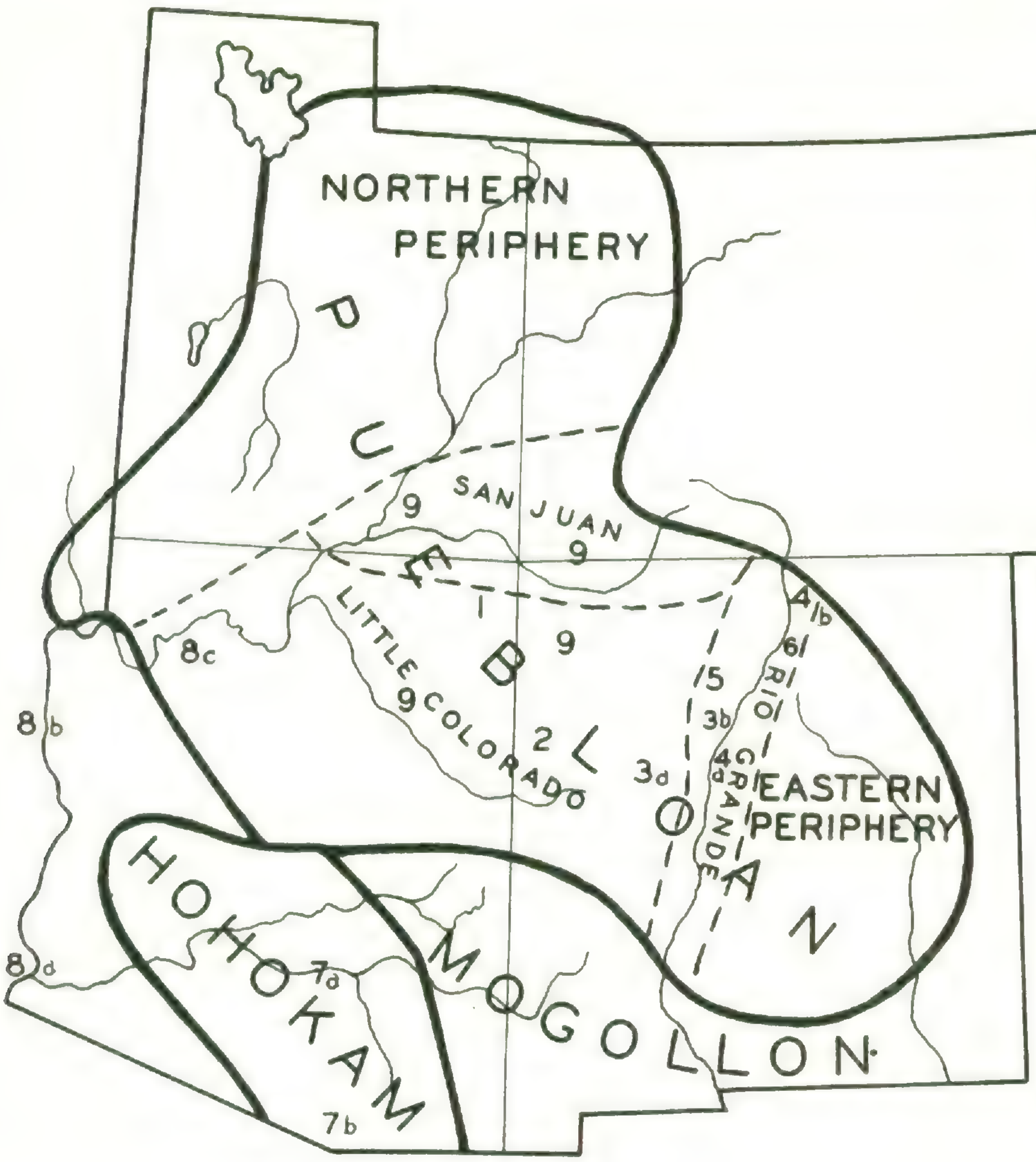
5. The Keresan Pueblos are all very similar and grow a type of maize which is roughly intermediate between the Pima-Papago and the eastern Pueblos. They might well have derived a good deal of their corn from the Hopi, an impression which is strengthened when the color is also considered.

6. The eastern Pueblos, considered as a whole, have about the same amount of Mexican complex as the Keresan, but show much more Eastern influence. As a whole, they are far from uniform and each one presents certain special features. By pueblos these are as follows:

San Ildefonso.—This pueblo shows a strong "Spanish" influence. This is not surprising since it is practically in the outskirts of the old Spanish-American town of Santa Fe. When the ears of our collections from the pueblo and from Santa Fe are laid side by side there can be little doubt that the San Ildefonso maize has been extensively mixed with that from the Spanish community near by.

Isleta.—With the exception of a few ears of maize, our collections from this pueblo are very similar to those from the western Pueblo area. This is to be expected since it is known that this pueblo took in a considerable number of Keresan-speaking refugees. Mrs. Parsons reports that clan structure shows resemblances to both Keresan and Tewa. Considering its location and its history it is therefore to be expected that the Isleta maize should include both types.

San Juan.—This maize is all very much alike and all similar to that from Mexican villages. This, too, is what might have been expected. The site of the first Spanish settlement in the Southwest, San Gabriel de los Españoles, is just across the river. The mission was succeeded by a little Mexican town, "Chamita", which was unfortunately on the right-of-



KEY TO MAP OF THE SOUTHWEST

ARCHAEOLOGIC AREAS

Puebloan area:
- San Juan—the early center of development.
- Little Colorado—area of early expansion.
- Northern Periphery—an area briefly occupied after 1000.
- Rio Grande—area of late settlement; present sites of most pueblos on river bottoms with irrigated fields a post-Spanish innovation.
- Eastern Periphery—late eastward expansion, abandoned in post-contact times.

Hohokam area: A major culture province of differing origin from the Puebloan.

Mogollon area: A major culture province of controversial origin; suspected of being Eastern in its derivation.

KEY TO TRIBAL AND LINGUISTIC GROUPINGS

Language group	*Pueblos or tribes*
1. Shoshonean	Hopi (First Mesa: Sichomovi, Walpi, also the Tewa village of Hano since about 1700; Second Mesa: Mishongnovi, Shumopovi; Third Mesa: Oraibi, Hotevilla, Bacabi)
2. Zuñian	Zuñi
3. Keresan	a. Laguna, Acoma b. Cochito, Santo Domingo, San Felipe, Zia, Santa Ana
4. Tiwa	a. Isleta, Sandia b. Taos, Picuris
5. Jemez	Jemez, Pecos, (Pecos joined Jemez, 1838)
6. Tewa	Nambe, Tesuque, San Ildefonso, San Juan, Santa Clara
7. Piman	Pima, Papago
8. Yuman	a. Yuma b. Mohave c. Havasupai
9. Athapaskan	Navaho, Apache

Notes.—Shoshonean was widely spread in the Great Basin, but only the Hopi took on Puebloan culture. Zuñian is of unknown relationship. The Tiwa, Jemez, Tewa, Keresan all belong in the Tanoan speech family, which is suspected to be of Eastern origin. The Piman are a northwest Mexican group. The Yuman seems to be an old group in the area. The Athapaskans are the latest entries into the area, probably beginning to drift in after 1000 A.D., and settling in between the Pueblos.

way of the railroad when it came through. The Mexicans accordingly moved across the river to join the pueblo of San Juan, which must have been in a depleted condition to have accepted recruits from outside the pueblo.

Taos.—While a larger collection from this interesting pueblo would be highly desirable, even our small collection seems to be significant. It includes some typical Pueblo types and two ears which are almost purely Eastern. This accords with the general Eastern cultural affinities of the Taos pueblo.

Tesuque.—While none of the ears from the pueblo are as purely Eastern as some of those from Taos, the general average is more Eastern than is that of any other pueblo. Tesuque is generally thought to be a Pueblo "shell" which was taken over by a non-Puebloan people. Judging from the maize alone, these newcomers might have been related to (or in close contact with) tribes of the Great Plains.

The significance of these conclusions must be placed against a background of southwestern culture history. There are in the Southwest two well-known basic cultures, the Anasazi and the Hohokam. There may well be a third, the Mogollon culture. The first two are the best known and our agricultural material relates to them. The Mogollon culture will, therefore, be reluctantly omitted from this discussion.

The two basic cultures with which we are concerned are the Anasazi and the Hohokam (see Map). Anasazi is a term used to refer to the culture which developed in the plateau region of the Southwest. The evidence to date shows that the Basketmakers, a non-agricultural people who had been living by hunting and gathering, learned of agriculture and began about 300 A.D. (or possibly earlier) to grow corn and squash *(Cucurbita moschata)*. These agricultural beginnings are usually placed in the "Four Corners" region (adjacent corners of Arizona, Utah, Colorado, and New Mexico). The crops and the idea of farming are clearly derived from some outside source.

Farming was apparently of minor importance in this early period. Crops may well have been handled as they were by some of the early historic Apache; i.e., corn and squash were planted and left to survive as best they could while the tribe went off hunting and gathering.

At a later period (500–700 A.D., Basketmaker 3) the Basketmakers show evidence of further contacts with some outside culture, and further cultural changes occurred. Pit houses were built (no houses are known from the earlier periods), pottery making began, and more varieties of corn appeared.

Around 700 A.D. more cultural changes occurred and sufficient new traits were introduced that a new cultural designation, Puebloan, is given. The first Pueblo period (700–900 A.D.) centered in the old Basketmaker area. Cultural developments, e.g., house types and pottery types, continued and further intro-

ductions of corn types are probable.

In each of these preceding periods there was some expansion outward from the central area in the "Four Corners" region. This expansion reached its maximum between 900–1100, Pueblo 2. People related to the Pueblo (Anasazi) culture then extended from southeast of Flagstaff, Arizona, nearly to Salt Lake, and in the latitude of the Grand Canyon reached from the Rio Grande to the Colorado. There was considerable regional variation. Large, many-roomed pueblos were already being built in the Chaco Canyon, the people of Mesa Verde were living in small masonry houses, while pit houses remained in use near Flagstaff. In all areas, however, the people were fully sedentary agriculturalists.

From the very beginning these people had been occupying a distinctly arid country and raising crops by dry farming. They must, therefore, have started with highly specialized crops. By locating their fields advantageously in reference to soil, run-off, and higher elevations with their greater precipitation they succeeded for hundreds of years in raising crops in areas considered impossible for modern agriculture.

In Pueblo 3 (1100–1300) a shrinking of the occupied area became apparent, and the settlements of small villages of loosely grouped houses now became compact towns of considerable size which were often built in defense locations. Houses were built wall to wall, several stories high, and entrance to first-floor rooms was normally from the roofs and not from the exterior ground level.

The attempted explanations for these happenings are still unreconciled. They fall into three categories. One theory points to climatic changes; another points primarily to the effects of invasion of nomadic peoples; a third to soil exhaustion and erosion. A variant of the second calls attention to the effect of warfare between the various Pueblo peoples.

That the distribution of corn types fits both the linguistic grouping and geographic position has already been pointed out. This may either imply that the various linguistic groups brought varying races of corn or that their geographic position in the Southwest gave them greater or lesser opportunities to get new varieties of corn. In view of the conservatism of the people, the high degree of adaptation of some of the earliest corn types, and the sudden appearance of new races of corn in special areas (e.g., dent corn of extreme Mexican type in the Northern Periphery), it seems fairly possible that the various linguistic groups represent different immigrations of people, each of which brought new agricultural material. The time of these introductions is not yet established, but further research now under way may do much to clarify this.

Perhaps a movement of peoples is implied in the first appearance of corn in the Four Corners region. Clearly, there were further introductions of corn types, between Basketmaker and Pueblo times. Whether other introductions occurred in the 600 years between Pueblo 1 and the "great drought" of 1300, or whether the next importation of corn came with peoples unsettled at the time of the "great drought" is not yet clear. It is even quite

possible that some of the corn types limited to the upper Rio Grande pueblos are post-Spanish, for after the Pueblo revolt some of the people are known to have fled into the Plains. It is not unlikely that some of these people later returned to the Rio Grande bringing new maize varieties with them, e.g., to Tesuque.

It is clear from the above that the development in the Anasazi area was a very complex affair involving different peoples and cultures. The cultural evidence suggests that in the thousand years after the introduction of agriculture there were repeated movements of people into the area. The implication of multiple introductions is particularly strong in the languages represented.

We are quite clear on the fact of multiple introductions of corn into the Pueblo area. We are sure of separate corn varieties appearing in Basketmaker 2 and again in the Basketmaker 3-Pueblo 1 periods; thereafter we can not yet place the time of arrival of the various races of corn that are modernly represented in the Anasazi area. Further work will surely make this possible.

The other basic culture of the Southwest to be considered here is the Hohokam. It is both less well known and less complex. Our knowledge concerning it begins about 600 A.D. according to Gladwin ('42, p. 4). At this time it seems already to have been a developed culture with agriculture and pottery, hence its true beginnings must go back of that date. The people lived in loose villages and occupied the middle Gila and the Salt River valleys, areas utilizable in their lower parts only by irrigation, whether from arroyo flooding or by using the waters of major streams. True canal irrigation was developed very early. Although we know less of this culture than we do of the Anasazi sequence, the evidence to date shows no such complexity in development as is found on the plateau. Culture periods are discernible but in the main they seem the result of local development with little outside influence until very late in their history.

The great problem of the Hohokam centers on their survival. After the mid-14th century the record becomes very incomplete. When the Spanish arrived they found the Hohokam area occupied by the Pima-Papago peoples. These latter were, and remain, village-dwelling farmers retaining the old Hohokam crops and some irrigation. The agricultural evidence suggests that they may be (at least in part) the descendants of the Hohokam.

A great contrast runs throughout these two cultures. The Anasazi developed into town-dwelling peoples with a tradition of masonry houses; they specialized in dry farming, developed elaborate rituals for rain, and had distinctive pottery techniques, etc. Their culture was complex, perhaps partially because of the different elements entering into its make-up. The Hohokam remained village dwellers with little evidence of alien peoples or cultures influencing them. They very early developed irrigation and established an elaborate ditch system in the Gila and Salt valleys. In religion, art forms, specific crops, and many more details they differed markedly from the Pueblo peoples.

This contrast is clearly reflected in the corn of the Southwest. Pueblo corn is complex in its make-up. It changes from period to period and to-day varies

throughout the Pueblo area. By comparison with the maize of other native areas, Hohokam maize is extremely uniform, probably one of the most uniform races one will ever find under primitive cultivation. It seems to have changed little in the past thousand years.

One of the crucial points in the relation of the Anasazi and the Hohokam revolves around the relationship between Basketmaker corn and Hohokam corn. We know Basketmaker corn from its preservation in the dry caves of the Southwest. It is closer to the Hopi and the Pima-Papago corn of to-day than it is to the maize of the Rio Grande pueblos. Some of the strains of Hopi corn fairly represent late Basketmaker corn. Hohokam corn is known from the published reports of the Snake Town plant materials (Castetter and Bell, '42), and from Haury's excavations in Ventana Cave (paper in press, 1944, but we have examined the maize remains). It is close to, though not identical with, the corn grown to-day by the Pima and Papago Indians. We have therefore a distinct race of corn in the Southwest that is common to the two different cultural areas at the earliest levels and which survives in part among the westernmost of the Puebloan peoples and among the desert-dwelling peoples who are presumably the descendants of the Hohokam.

We have not yet photographed and measured all the prehistoric North American corn available in museums and private collections. Until that job has been done an extended discussion is premature. However, from the prehistoric maize we have already seen and the junior author's studies of modern Mexican maize, it seems probable that there were at least three waves of prehistoric maize in North America. The first was a small-cobbed, small-seeded, tesselated, compressed, undented race. It survives to-day in its purest form in the maize of the Pima and Papago. It can be seen only slightly mixed in the early varieties of the Pawnee and other Missouri River Indians, in old varieties from the Gaspé Peninsula in Canada, and in *Maiz reventador* and related varieties from western Mexico. Its presence in Oaxaca, Mexico, in 400 to 600 A.D., is suggested by the representations of maize on the funerary urns of the Zapotecs, which resemble Basketmaker maize more closely than they do the modern varieties of Oaxaca. It is one of the types recovered from mounds and other archaeological sites in the Mississippi Valley (where it is apparently one of the earlier types to appear, though the evidence is not consistent on this point). The other two waves we have already described as "Mexican" and "Eastern." In later papers we hope to be able to work out the order of their appearance and perhaps ultimately to trace them back to their origins.

SUMMARY

1. The technical advantages of *Zea Mays* for cytological, genetical, and archaeological study are described. It is concluded that when we eventually combine the information from these three disciplines we shall have a more complete picture of maize in space and in time than will ever be possible for any other

world crop.

2. Because of its geographic and climatic isolation, southwestern maize is simple in its variation pattern as compared with that of Mexico or Guatemala.

3. The importance of collecting samples from remote and relatively conservative Indian communities is discussed.

4. One of the most significant maize characters in the Southwest is the denting of the kernel due to a cap of soft starch. Its genetics is obscure but a large number of genes are involved. Much of the denting of Indian varieties in this area is of such slight grade that it might readily be overlooked. Breeding experiments have proved that even the slight grades of it in that area are genetically controlled and are not merely due to harvesting when immature.

5. Maize in the Southwest has come from at least four different sources: the Basketmakers, the Hohokam, the Mexican plateau, and eastern North America. The maize of the Basketmakers and of the Hohokam was very similar but apparently distinct.

6. In the Southwest the following three characters of the maize ear are correlated: ear taper, row number, and denting of the kernel. They are referred to as the Mexican complex of characters. Kernel width, shank diameter, an enlarged butt to the ear, and straight rows are also correlated. They are called the "Eastern" complex.

These eight characters and two others were used in constructing two indices for measuring southwestern corn. Plotted on x and y axes they form a "comparison grid" on which scatter diagrams of the maize from one pueblo may be compared with the maize from another, or on which the average values of different collections may be similarly compared.

7. Scatter diagrams are presented for a portion of our collections of modern maize and a summary of the conclusions drawn from them is given on pages 309-312.

8. An attempt is made to interpret these findings in terms of what is known about the history of the Southwest from the beginnings of agriculture to the present. The following hypotheses seem fairly well established:

A. The Basketmakers and Hohokam brought similar, though non-identical, strains of maize into the area. The Hohokam maize apparently was brought up the west coast of Mexico and has remained with only slight modifications as that now grown by the Pima and Papago.

B. The maize of the early Basketmakers was progressively modified more and more in the direction of the maize of the Mexican plateau, causing an increase in row number and in the amount of denting of the kernel.

C. The maize of the Southwest was greatly modified from 1200 to 1300 A.D. The complex of characters introduced at that time is characteristic of the eastern United States and the easternmost pueblos were most affected and the westernmost the least.

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Explanation of Plates

Plates 2–4 are from the special collection of maize photographs assembled in the Museum of Anthropology of the University of California at Berkeley. We are indebted to E. W. Gifford for permission to reproduce them. All three illustrations were photographed, printed, and reproduced at approximately natural size.

PLATE 2

Papago white flour corn. Three ears purchased from Mrs. Margaret Harvey (Xavier) at Choulick, near Sells, Arizona, on the Papago reservation, by Edgar Anderson and Emil Haury. In the Anderson sollection they are Choulick Nos. 1–3, from left to right in serial order. Their scores on the two indices used in this paper are as follows (in each case the Eastern Index is given first, followed by the Mexican Index): —2, —1; —2, —2; —2, —2.

CARTER & ANDERSON—MAIZE IN THE SOUTHWEST

EXPLANATION OF PLATE

PLATE 3

Hopi white flour corn. Right, Carter No. 497, left, Carter No. 498. The former scores 1, 2 on the Eastern Index and 1-2 on the Mexican Index. The latter scores 2, 3. Note the faint crease or "dent" at the apex of some of the kernels. This degree of denting is commonly found in Hopi flour corn.

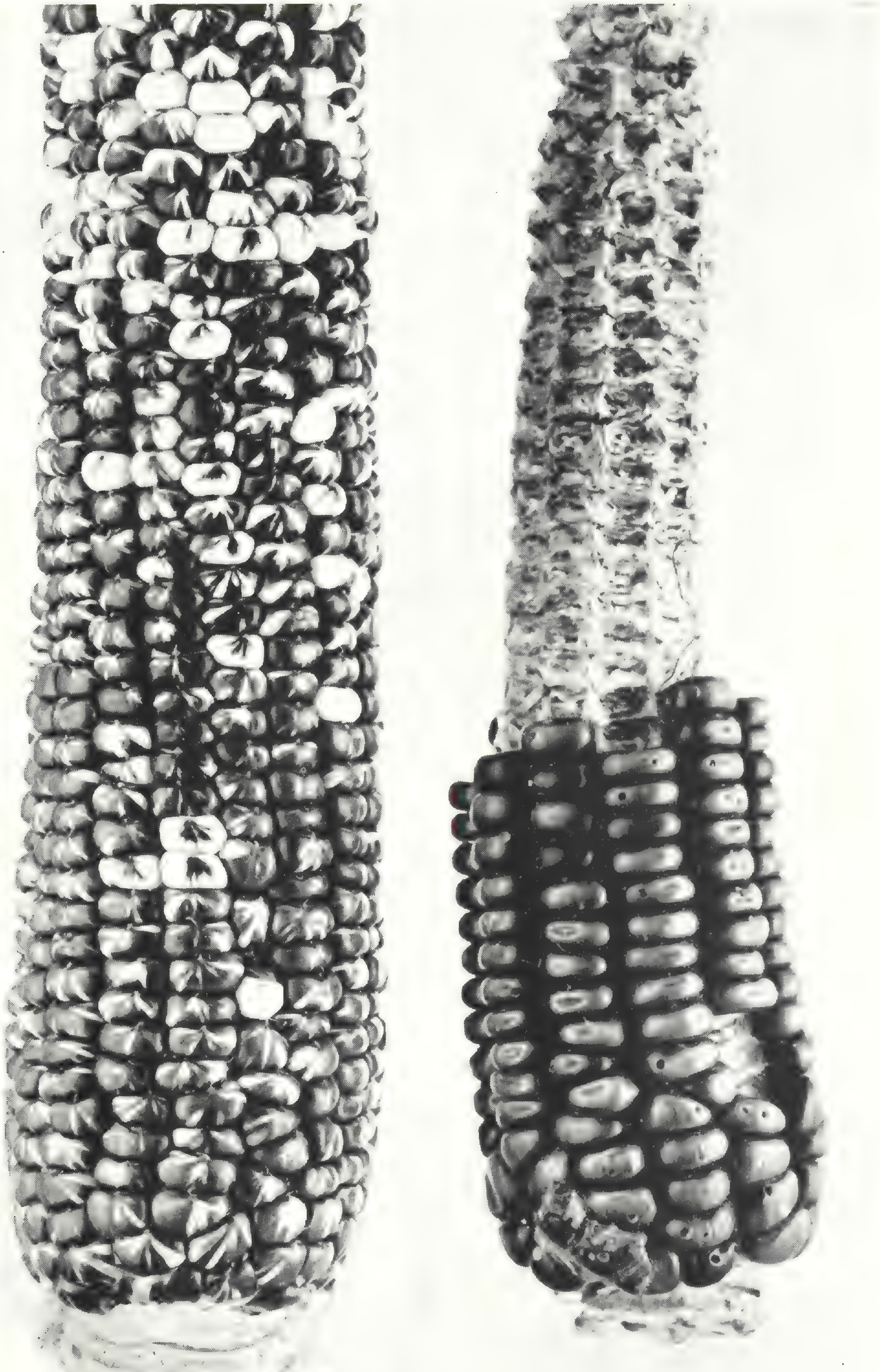
CARTER & ANDERSON—MAIZE IN THE SOUTHWEST

EXPLANATION OF PLATE

PLATE 4

Right, Isleta red corn. Carter No. 315, scoring 6½ on the Eastern Index and 2½ on the Mexican. Note the wide seeds and the straight rows of uniform kernels.

Left, Tesuque red-and-white corn (mosaic pericarp). Carter, scoring 4 on the Eastern Index and 5 on the Mexican. At the base of the photograph note the heavy, well-developed shanks which support the ears. These are characteristic of much modern Pueblo corn but did not appear in most of the Southwest until after 1200 A.D.



CARTER & ANDERSON—MAIZE IN THE SOUTHWEST

CONTRIBUTIONS TO OUR KNOWLEDGE OF AMERICAN CARBONIFEROUS FLORAS

VII. Some Pteridosperm Stems From Iowa

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The occurrence of richly fossiliferous coal balls in the Upper Pennsylvanian rocks of Iowa has been reported by Darrah in 1939 and 1941. In the latter paper he gives a list of the species observed as well as a brief discussion of the dominant floral elements. The distribution and occasional abundance of these petrifactions in Iowa is now sufficiently well known so as to render a detailed review unnecessary, but certain introductory remarks are in order relative to the general floristics, as compared with the Illinois coal balls.

In September, 1944, Mr. Frederick O. Thompson of Des Moines very generously turned over to the writer, for study and preservation in the Henry Shaw School of Botany, a selected portion of his remaining coal-ball specimens. All of this material was collected from the highly prolific, although now abandoned, Urbandale mine located on the north side of U. S. Highway No. 6, 1.2 miles west of the city limits (63rd Street) of Des Moines. The coal seam from which the petrifactions were obtained lies 185 feet below the surface, the elevation of the latter being 805 feet. It is regretted that a more precise stratigraphical position, other than "the Des Moines Series," cannot be given at this time. The author has been informed that a detailed study of the stratigraphy of this region will be published shortly, and it is hoped that this information may be given in the next number of our "Contributions" which will deal with seeds from the Urbandale coal balls.

Our collection consists of approximately 110 cut slabs which vary from 3 to 20 cm. in diameter. It is admittedly small as coal ball collections are accounted, yet rich in the number of seeds, leaves, pteridosperm stems, fertile fern-like foliage, and other plant parts, many of which are either striking novelties or very imperfectly known.

During the three years prior to the war the paleobotanical work in this laboratory was devoted very largely to a study of fossil plants from certain southern Illinois mines.[1] Although the progress made in that region constitutes but little more than a bare introduction to the field work lying ahead, it does seem clear that the Carboniferous flora that occupied much of southern Illinois was dominantly pteridophytic. Certainly the Herrin coal from the great Pyramid strip mine south of Pinckneyville was formed to a very considerable extent from the remains of *Lepidodendron* (Pannell, '42), and the roof shales above the same coal in the

[1] See parts I–VI of this "Contributions" series, in Ann. Mo. Bot. Gard. **29-30**. 1942-43.

Issued September 15, 1945.

Old Ben mine #11, in Franklin County, display a preponderance of arborescent lycopods, articulates, and ferns.

Lepidodendron is so preponderant in the Pyramid mine coal balls that we have on numerous occasions all but decided to abandon further collecting there, yet the constant lure of fragments of other plants has brought us back time and again, and it is very probable that this will continue for some time.[2]

It seems reasonably safe to express the opinion that the study of coal balls is a phase of paleobotany that promises to be long and productive. It is only in England that anything approaching exhaustive studies has been carried on, and even there we have no reason to assume that the task is completed. The works of Williamson and Scott, and a number of other British paleobotanists of Scott's period, were based on coal-ball petrifactions, yet few of these investigators were collectors. If we may judge from Scott's written works and his magnificent slide collection, his source of supply was primarily through the medium of professional collectors and other persons. Thus only the more striking and obviously new things reached his hands. Perhaps it would be asking too much to expect one man to contribute more, yet I cannot help but feel that Scott's contributions would have had an even more vital effect on the following generation had he personally supervised the cutting of some few tons of coal balls in his own laboratory.

Judging from our own experience in Illinois and from Mr. Thompson's vast collections, most of which are deposited at Harvard University, it seems clear that large-scale collecting is the primary requisite to the restoration of reasonably complete plants and their assemblage into correspondingly complete paleo-landscapes. Studies of the flora of an individual coal ball are certainly a thing of the past. Productive mines must be revisited many times and collections considered in terms of tons. This may savor of mass production rather than "scientific procedure," yet it is the only way in which scattered parts of the plants may be brought together and those plants assembled into representative restorations.

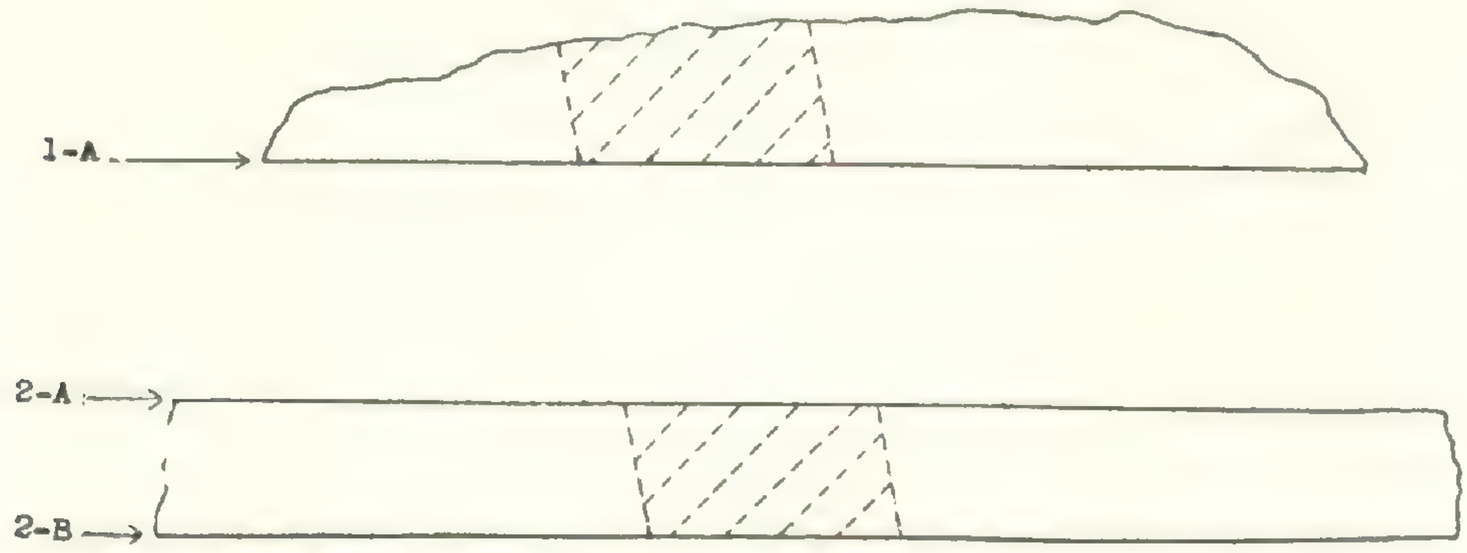
To return to the Pyramid coal balls, evidence of seed plants in the petrifactions from that mine are meager indeed. Apparently they were not abundant in the immediate environs, and the Urbandale specimens from Des Moines thus present a most striking contrast. Although fern-like fructifications are present in some abundance the Lycopods are comparatively rare, their place being taken by an extensive array of seeds, Cordaitean stems and leaves, and stem-remains seemingly referable to the Pteridospermeae. From this assemblage the latter have been selected for primary consideration.

Medullosa Thompsonii sp. nov.

The following description of this stem is based on two slices cut from a coal ball approximately 15 cm. in diameter, one being an end cut. Both are approximately 1.5 cm. thick, and the stem passes through both pieces. Between these two

[2] In a collection of coal balls obtained from the Pyramid mine in December, 1944, one contains numerous specimens of *Cordaianthus* which is, judging from a preliminary study, closely related to *C. Shuleri* Darrah. In view of the frequent occurrence of *Mesoxylon* it is not surprising that *Cordaianthus* should have turned up, although this is the first record of it from that region so far as I am aware.

portions of the original coal ball, one or two slices, apparently totaling a little more than 3 cm. in thickness, have been removed. Mr. Thompson, the collector, has informed me that this intermediate portion was one of the specimens sent to the Botanical Museum at Harvard University some few years ago which have not been available for study. There is, however, no doubt as to the relationship between the two pieces in our collection.



Text-fig. 1. Sectional view through coal ball WCB420, with approximate position of *Medullosa Thompsonii* stem shown in shaded area; 1-A represents face A of block 1, and 2-A, 2-B represent faces A and B of block 2. Reduced about one-half.

Aside from its other significant features, this stem (and the remains of other Medullosan stems, petioles, roots and leaflets in associated coal balls) is of special interest in that it demonstrates stelar fusions more clearly than has been reported in any previously described species of the Anglorota (Schopf, '39) group. Text-fig. 1 shows the order in which the preparations were made.

The Stem and Leaf Bases.—

The stem measures approximately 4.3 x 3.0 cm., exclusive of the petiole base shown at the left in fig. 1. It is slightly crushed and must have been cylindrical, or nearly so, measuring about 3.6 cm. in diameter in life. It consists of three sharply defined regions: an outer fibrous cortex (rind) which certainly contributed appreciably to the support of the plant, a broad inner parenchymatous cortex, and a central stelar system bounded externally by a band of periderm. Every section also displays the bases of at least one or two petioles in various stages of departure. It is possible to distinguish sharply between petiole base and stem only where the rind is in the process of developing between the two. Thus in fig. 1 the partitioning fibrous tissue is shown developing at point *a*, preparatory to the departure of the petiole at the left.

Those who are familiar with the Medullosas will observe an unmistakable similarity between this stem and the English *M. anglica*. A number of differences will be pointed out in the course of the description which, it is believed, adequately justify the distinct specific designation.

The rind is bounded by an epidermis consisting of brick-shaped cells slightly elongated radially (fig. 32). The radial, and especially the outer walls, are somewhat thicker than the inner ones. This is probably accounted for, in part, by the former presence of a cuticle, although it has not been possible to distinguish this from the wall of the epidermal cells.

The cortical cells, which are small and nearly isodiametric immediately within the epidermis, gradually increase in size, attaining a maximum within the fibrous zone. In *Medullosa anglica* the outermost cortical layer is illustrated (Scott, '99, fig. 13) as being palisade-like after the fashion of the epidermis. No such differentiation of the outer cortex is present in the Iowa specimen. In cross-section the fibrous strands (fig. 5) are circular to slightly elongate radially. These anastomose, although only at very extended intervals, and appear in longitudinal section as very nearly parallel strands. An especially noteworthy feature of this part of the stem lies in the rarity of associated secretory canals. They are present deeper within the cortex although markedly less conspicuous than in *M. anglica* and other related species.

It may be noted that in fig. 1, from approximately points *b* to *c*, the fibrous strands are lacking. The rind is replaced in this region by a periderm (fig. 33) consisting of an outer uniform layer of phellem 4 to 5 cells deep; within this there is a somewhat broader band of phelloderm of less regularly aligned cells which merge with the larger cells of the cortex within; and in between these two tissues lies what apparently was the phellogen. These terms are used as they are conventionally applied to living plants on the assumption that they correspond to the respective tissues of a normal periderm. In his description of *M. anglica,* Scott mentions a similar "distinct interruption of the hypoderma cortical fibers between the bases of adjacent leaves," and he suggested that it was in this region that the adventitious roots were inserted. While it is not possible to confirm this with *M. Thompsonii* it seems very likely that such may have been the case. Although Medullosan roots are abundant in other associated coal balls none were found in organic connection with the stem. This might be explained by the fact that our specimen probably represents a fragment of the plant from some distance above the ground.

The internal or stelar periderm differs in no way from that of other related species. At the level of the stem represented by surface A of block 2 it encloses three steles, lettered *x*, *y*, and *z* in fig. 2. The ground tissue immediately surrounding the steles is not sufficiently well preserved to merit further consideration. The steles vary with respect to size and development of secondary xylem. Stele *x* measures approximately 7.0 x 3.5 mm. and is somewhat crushed; stele *y* is 2.0 mm.; and stele *z* is 6.0 x 3.0 mm. in diameter.

Like the periderm, the primary wood seems to present no characters of special distinction. The secondary wood, however, is notably endocentric, being (Schopf, '39, p. 203) weakly developed towards the outside (fig. 2). Although the relative arrangement of the three steles has been slightly distorted, the position they

occupy in block 1, where all three are fused together (fig. 3), is the same, indicating that their position as illustrated in figs. 1 and 2 is but little changed from that in life.

By far the most interesting feature of this *Medullosa* stem lies in the fusion of the steles between the A surfaces of blocks 1 and 2. It may be noted in fig. 3 that stele *y* has clearly united with *x* while the union of *z* with the *x*-*y* fusion is in its initial stages.

The leaf traces depart from the steles as comparatively large (slightly less than 1 mm.), nearly cylindrical masses of xylem (fig. 14a). The initial trace almost immediately starts to divide (fig. 14b), forming the numerous collateral bundles (fig. 5) scattered through the massive parenchymatous cortex between the periderm and rind. Where the bundles are still in close proximity to the steles they are usually enclosed by a distinct sheath, although this is not as strongly developed farther out in the cortex.

An especially distinctive feature of M. *Thompsonii* is the comparative paucity of fibrous tissue in association with either the bundles or the secretory canals. Conspicuous sclerenchymatous sheaths or strands such as are figured for M. *distelica* (Schopf, '39, fig. 5) and M. *Noei* (Steidtmann, '44, pl. VI, fig. 1; pl. VIII, fig. 1) are not found in M. *Thompsonii*.

Discussion of Stelar Anatomy and Comparison with Other Species.—

Stelar fusions and stelar branching were evidently frequent in the stems of the highly complex Permian species (Seward, '17, p. 104). That the same events take place in the structurally simpler species of the Anglorota group is known only in a very sketchy way. In her description of M. *centrofilis*, De Fraine noted the fusion of two steles, but at the point of fusion the steles are fragmentary (De Fraine, '14, pl. XV, fig. 2), and it was not possible to illustrate clearly the phenomenon. Scott referred to an interstelar fusion in M. *anglica* but here again the preservation was imperfect.

It is important that we have a clearer knowledge of this phase of *Medullosan* anatomy, partly in order to evaluate correctly the taxonomic use of stelar number and size, and partly to aid in interpreting phylogenetic trends in the group.

Professor Bower's investigations in the ontogeny of the ferns, the mature stelar systems of which are often rather complicated, indicate that the steles invariably started as a single protostele. It is hardly conceivable that the stelar ontogeny of the Medullosas could have followed any other course.

Numerous writers have compared the individual steles of *Medullosa* with the single stele of *Heterangium*. That portion of the stelar system shown in fig. 3 presents a rather close approximation to the *Heterangium* type. Whether or not the fusion of stele *z* with the already fused *x* and *y* steles became complete, resulting in a more nearly cylindrical (single) form, cannot be determined since the stem passes out of the coal ball shortly beyond the point illustrated. Even though the stele should assume a perfectly cylindrical form at a higher level there is, of

course, no implication that it should be referred to *Heterangium*.[3] There is no doubt, however, that such a stem fragment, if found isolated, would present a striking resemblance to that genus, at least as far as the stele is concerned. Inasmuch as the seedling of *M. Thompsonii* almost certainly started with a single stele it seems likely that divisions and fusions occurred rather regularly through the length of the stem.

A number of reviews dealing with Medullosan relationships have appeared during the past few decades, and there is no cause for adding to this literature at present except for the Anglorota subgenus. This subgenus was created by Schopf in 1939 with *M. anglica* as the type species. Other species that may be included are *M. centrofilis* De Fraine, *M. pusilla* Scott, *M. distelica* Schopf, *M. anglica* var. *thiesseni* Schopf, *M. Thompsonii* Andrews, and the *Medullosa* illustrated in fig. 7 (see p. 335). There can be no doubt that these constitute a very closely related assemblage, distinct in certain seemingly valid characters, yet similar enough to justify speculation concerning their interrelationships.

Before attempting any such racial considerations it is very necessary to determine in so far as possible what characters may be of taxonomic value and what ones are too variable within an individual specimen. In order to facilitate comparison of the above seven species they are shown, all at the same magnification, on plate 12. It is evident that their structure is pronouncedly similar in spite of the considerable size difference between the large *M. anglica* and the comparatively minute *M. pusilla*. The group is a remarkable one from the viewpoint of structure and certainly a closely related taxonomic unit, and there is no longer any doubt that it was widely distributed during the Pennsylvanian. Whether the species names that have been used to designate these stems would stand if the entire plants were known is, of course, not now possible to determine. In any case, the answer would not detract from their interest, and temporarily at least they have been handled in the most expedient way.

It seems unlikely that quantitative differences, such as size of the individual steles and the number composing a stem, can be allowed to bear much taxonomic weight. In *M. anglica* alone the steles are reported to range from 6 to 30 mm. in diameter and the almost continuous range throughout the group is clearly shown in figs. 23–30. Knowing the wide variation that may exist in both primary and secondary stelar bodies of fossil and living plants (Bower, '30; Pannell, '42), such characters must obviously be regarded with considerable deliberation. Neither is the number of steles in itself an entirely dependable character. Three is apparently the "typical" number in *M. anglica*, *M. Thompsonii*, *M. anglica* var. *thiesseni*, and *M. pusilla*, and it is more than likely that fusion took place in the stem of *M. centrofilis* resulting at certain levels in three steles.

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[3] It should perhaps be shown that we are dealing with a fusion here and not a division; *i. e.*, that the sequence is being read in the proper direction. Although the stem fragment is short it is clear that the petiole shown in fig. 1 (from which fig. 2 is magnified) is in a more advanced state of departure some 1.5 cm. farther along the stem (from the point at which fig. 3 is taken). This is indicated by the more nearly complete development of the rind separating the petiole from the stem.

De Fraine in her discussion of the affinities of *M. centrofilis*, notes that "the agreement in practically every detail between the steles of the specimen and those of *M. anglica* and *M. pusilla*, leave no doubt as to its very close relationship with those fossils." The relative extent of endocentricity in the steles of different species may present a character of value; at least *M. distelica* seems to display this to a more marked degree than the other species. Whether or not this varies appreciably through the course of an individual stem remains to be determined when longer specimens are discovered.

Aside from these variable characters of the stelar system itself, considerable weight has been attached to the presence or absence of secondary tissues accompanying the leaf trace on its departure from the stele. This secondary wood is conspicuously abundant in *M. anglica* but was reported absent in *M. pusilla* and *M. centrofilis*, and absent or very weakly developed in *M. distelica*. It is likewise absent in *M. Thompsonii*. As far as the leaf traces themselves are concerned they appear to present a very marked uniformity in all of the species described above. The nature of the accompanying fibrous sheath may, however, be of taxonomic value.

Medullosa Thompsonii differs from *M. anglica* in the comparative rarity of secretory sacs in the cortical rind, as well as the parenchymatous middle cortex. The hypodermal cells in the two are likewise divergent in their structure (c. f. Scott, '99, fig. 13; and fig. 32 of this paper). Although the Iowa stem and *M. anglica* are undoubtedly closely related the differences that have been noted seem to justify segregation.

The differences in number, size, and extreme endocentricity of the steles, as well as abundant development of secretory sacs in the cortex, clearly set *M. distelica* apart from the Iowa specimen. Of the figures in plate 12, this leaves only *M. centrofilis* and *M. pusilla*. If size has any significance in classification the latter certainly deserves its own pigeon-hole, and the secretory canals are reported by Scott as numerous.

Although they are interesting and illustrate the size range in the Anglorota group I do not feel that either *M. anglica* var. *thiesseni* or *M.* sp. (fig. 7) is sufficiently well preserved to allow of precise comparison. *M. anglica* var. *thiesseni* and *M. Thompsonii* may very possibly be one and the same species but this cannot be verified until supplementary material of the former is forthcoming, showing well-preserved extra-stelar tissues.

Thus of the previously described species of *Medullosa* which present sufficiently well-preserved detail to allow of precise comparison, *M. centrofilis* appears to be the most closely related to the Iowa fossil. The differences are not great, the central "star-ring" and the secretory canals in the cortex of *M. centrofilis* being the only conspicuous points of divergence.

Leaves.—

Numerous isolated petioles or rachis fragments belonging to Medullosan stems

are present in the Urbandale coal balls. They are not only abundant but represent, as well, different branching orders of the leaves, as is clearly evinced from the size variation and the anatomy of the rind.

From a study of the literature and the material at hand, it appears that the following characters are most significant in the classification of these fossils: structure of the fibrous strands composing the rind; presence or absence of the secretory ducts, their distribution, and whether or not they are regularly associated with the fibrous strands; distribution of the vascular bundles. Use of the last character is restricted to those specimens in which there is no appreciable amount of crushing or distortion.

A number of the better-preserved specimens agree closely enough with the attached petiole bases of *M. Thompsonii* to warrant their inclusion under that species. These isolated petiole and rachis remains[4] (figs. 9, 12, 13, 19) vary from 16 mm. (fig. 9) to a little over 4 mm. (fig. 12) in diameter. The diameter of the leaf base shown about to depart from the stem in fig. 1 measures approximately 2.2 cm. in diameter. In all probability these leaf bases tapered abruptly outward during the first few centimeters and then only very gradually to the extreme tip of the leaf, as in modern cycads and large-leafed ferns such as *Cibotium*. Figures 13 and 19 may be taken then as representing the rachis[5] at a point some distance from the base of the leaf, and fig. 12 in turn may represent a distal secondary rachis or a tertiary one.

In the upper right portion of fig. 9 there is a localized group of a dozen or more conspicuous secretory canals scattered through, and inside of, the rind. With this exception, however, the petiole specimens referred to *M. Thompsoni* present a relative paucity of secretory canals. The lack is certainly more pronounced than in previously described species and compares closely with the similar negative feature of the leaf bases in organic connection with the stem.

One of our coal ball specimens contains a number of leaves of the *Alethopteris* type, most of which have been cut in transverse sections. Although there is considerable reason to believe that certain species of this genus were borne by the Medullosas it must be admitted that the present report does not further our knowledge of that relationship. However, certain features of these Iowa specimens do contribute toward a clearer understanding of their structure.

The restoration shown in pl. 13 is a composite drawing compiled from the best-preserved portions of a dozen or more leaflets. The coal ball containing the pinnules is highly pyritized, although that mineral has not penetrated the leaf tissues to any appreciable extent. Both peel preparations and ground sections proved unsatisfactory for photographic reproduction.

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[4] It may be noted that the illustrations of these petiole and rachis fragments in transverse section are all reproduced at the same magnification. Although it necessitates considerable variation in figure size I believe that if this practice could be more generally adhered to it would greatly facilitate the distinction between different species as contrasted with the differences represented by the ordinal position of the leaf part represented.

[5] In view of the rather irregular dichotomy of the frond of *Alethopteris* and certain related species (Bertrand, '32) the term rachis is used here rather loosely to signify the larger proximal branches of the petiole.

Within the epidermis there is a clearly defined hypodermal layer and under this a third seriation of cells differing from the hypodermal in their vertical alignment. In all probability they were chlorophyllous in life, functioning as palisade cells.

The under-side of the leaflets presents a conspicuous combination of short papillose cells and long multicellular hairs. The cells densely cover almost the entire under-side, and in all probability the stomata were located among their swollen bases. It has not been possible to identify the guard cells. The elongate multicellular hairs are most striking (fig. 11). Most of them have been broken, but the few apparently entire ones consist of five or six cells. Within the epidermal papillae there is a zone of compact, more or less isodiametric, angular cells. The tissue between this and the palisade cells is very poorly preserved. However, I have observed that the region in close proximity to the central vein is occupied by loosely organized, horizontally arranged cells. There is no reason to believe that these did not extend to the margin of the leaflet as shown, although it should be noted that this feature of the restoration is uncertain. Nor has it been possible to distinguish with clarity the structure of the vascular tissue.

Sections of presumably Alethopterid leaves found associated with Medullosan petioles have been described and figured by Scott, '99, Steidtmann, '44, and Schopf, '39. Scott (p. 101) notes: "I have not yet found absolute proof that these leaflets belong to the '*Myeloxylon*' rachis, but the constancy of association, and general agreement in the structure leaves no real doubt." This opinion seems to be shared by Schopf and Steidtmann in their respective treatments of *M. distelica* and *M. Noei*.

The general similarity among the leaves illustrated by Scott and Steidtmann and our Iowa specimens lends support to this relationship with the *Myeloxylon* petiole remains. The distinctive multicellular hairs are illustrated in Steidtmann's specimens although these are not indicated in the English material. Nor is the hypodermal layer of cells indicated in Scott's fig. 17, although the preservation of his material apparently was rather inferior in this respect.

There is also a striking resemblance between the anatomy of the fossil leaflets and those of the living cycads (fig. 10). Assuming that the elongate spongy mesophyll cells are uniform through the width of the fossils the two are very nearly identical, with the exception of the multicellular hairs that are lacking in *Cycas revoluta*.

Roots.—

A number of isolated roots have been found in the Urbandale petrifactions. Many of them compare closely enough with those described by Steidtmann ('44) and Schopf ('39) as to leave no doubt of their Medullosan affinities. It is not possible, however, to refer them with certainty to any species of the genus.

Most of the roots are tetrarch (fig. 15) and show various stages in the development of the secondary xylem. One is hexarch (fig. 16) although the stele is enclosed by a band of periderm characteristic of Medullosan roots.

The Restoration (pl. 13)—

The conspicuous role that the Medullosas evidently played in Pennsylvanian landscapes makes some sort of restoration of these plants desirable. Although the plant shown in pl. 13 is captioned "a *Medullosa* of the Anglorota group" it is based primarily on the stem and leaf remains of *M. Thompsonii.* However, aside from differences in size, there is no reason to believe that the Anglorota species presented any great dissimilarity in their general habit. In order that this restoration may bear no false implications or in any way convey impressions that do not rest on established facts the following points should be clearly understood: We do not know the exact height of any species of this group, but judging from the relatively small amount of supporting tissue they were probably not more than a few feet high,—perhaps 3–5 feet is self-supporting. If the plants attained greater heights let us assume up to 15 or 20 feet, it is likely that they grew in rather dense stands and supported one another, or relied upon the trees and shrubs of other species.

The fronds were large in proportion to the diameter of the stems, and in order to arrive at a reasonable approximation of their size, measurements have been made of ten living species of cycads as well as a species of *Cibotium* growing in the Garden greenhouses. In the cycads there is a rather constant ratio between the basal diameter of the petiole and the length of the leaf. However, Alethopterid leaves possessed a considerably higher breadth/length ratio than modern cycads, probably lying closer to that of a *Cibotium* frond. Yet from the structural similarity of the *Alethopteris* leaflets and those of a modern cycad it is certain that the weight of a *Medullosa* leaf, for its total area, was much closer to the cycads than a large-leafed fern. These points have been duly considered in determining the size of a *Medullosa* frond, relative to stem diameter, based on petiole diameter measurements.

As for the morphology of the leaves, indications are that the primary rachis gave rise to successive unequal bifurcations in at least some species of *Alethopteris* and related form-genera (Bertrand, '32, p. 67; Kidston, '11, fig. 7). It is reasonable to assume that the leaf size varied appreciably in the different species of the Anglorota group. Although the stem as shown in the restoration probably bore a crown of perhaps six to a dozen (more or less) leaves more precise detail could be shown if only one leaf were drawn in. Furthermore, the leaves were probably borne in a manner comparable with that of modern tree-ferns although the single leaf shown has been illustrated so as to conserve space and at the same time bring out the salient features of its construction.

Reproductive structures have been omitted from the drawing. Although seeds have been found on *Alethopteris* leaves (Halle, '29) and they are abundantly associated with the stems in the Iowa coal balls, it seemed best to postpone this feature of the restoration until further evidence is forthcoming.

Diagnosis of Medullosa Thompsonii Andrews.—

Stem approximately 3.6 cm. in diameter, epidermal cells radially elongated,

fibrous cortical strands circular to slightly elongated radially and anastomosing only at extended intervals, secretory canals rare in outer cortical (rind) region, internal periderm present; stelar system of three endocentric steles fusing upward, leaf trace sheaths poorly developed.

Petioles approximately 2.2 cm. in diameter at point of departure from stem, secretory canals not abundant.

Locality: Urbandale Coal Mine, Des Moines, Iowa.

Horizon: Pennsylvanian, Des Moines Series.

Type specimen: No. WCB420, Henry Shaw School of Botany, paleobotanical collections.

Other Medullosan Stems.—

Fragments of other stems, recognizable as belonging to the genus *Medullosa,* are included in our collection from the Urbandale mine. Although not sufficiently well preserved to merit specific recognition, one is worth a brief note. As shown in fig. 7 only the steles, of which there are two, are preserved. The periderm is sufficiently intact to indicate that no more than two were originally present. Aside from this, all extra-stelar tissues have been destroyed. The smaller stele measures 8.0 x 4.0 mm., and the larger 15.5 x 4.0 mm. although it is obviously crushed, probably nearer 15.5 x 6 mm. in life. Both are slightly endocentric, but this feature is much more pronounced in *M. distelica,* the only other known bistelar species. It is also appreciably smaller than *M. distelica* (see pl. 8), being more or less intermediate in size between that species and *M. Thompsonii.*

Myeloxylon Bendixenii sp. nov.

Included in the Medullosan petiole specimens from the Urbandale mine is one that is decidedly different from those described above as *Medullosa Thompsonii.* This petiole is large, as compared with the others, measuring approximately 3 cm. in diameter although it was somewhat compressed prior to fossilization (fig. 8). Since the most distinctive features lie in the structure of the rind a representative portion of this is shown in detail in fig. 35. Within the epidermis there is a band of parenchymatous cortex, 5–6 cells deep. This in turn encloses a broad and very conspicuous fibrous zone which averages nearly 2.0 mm. thick. The radial and tangential dimensions of the fiber bundles are more or less equal although many of them are quite irregular. A glance at fig. 8 reveals more in this respect than could be conveyed by many pages of measurements.

With the exception of the outermost strands almost every strand is associated with a secretory duct on its outer side (fig. 35). These ducts average 190 μ in diameter.

As may be noted in fig. 8, there are a few fibrous strands scattered deep within the petiole. The secretory ducts, with their conspicuous black contents, are also numerous in the ground parenchyma scattered among the vascular bundles. The large size of this petiole, as well as the anatomical details of the rind, leaves little doubt that it is not to be associated with *Medullosa Thompsonii.* Thus, until more

stem remains are retrieved from Iowan petrifications it must be referred to *Myeloxylon.*

A comparison with other American species reveals no close alliance with previously described specimens. Penhallow's *Myelopteris (Myeloxylon) topekensis* (Penhallow, '97) is not sufficiently well preserved to allow comparison. Arnold and Steidtmann ('37) proffered the same opinion concerning this species, and there seems to be no justification for further reference to it in the literature, since its exact origin is also not known.

Myeloxylon Bendixenii differs from both *M. missouriensis* Arnold & Steidtmann ('37), and *M. zonatum* Steidtmann ('44) in the following respects: the rind of *M. Bendixenii* is twice as thick; secretory canals are much more abundantly associated with the fibrous strands; and the inner border of the rind is not as sharply delimited.

Diagnosis of Myeloxylon Bendixenii Andrews.—

Petiole large (3 cm. diam.), parenchymatous cortex 5–6 cells deep between epidermis and rind, rind (fibrous zone) 2 mm. thick radially, fiber bundles more or less isodiametric in transverse section, almost every bundle accompanied by a secretory duct on outer side, ducts averaging 190 μ in diameter.

Locality: Urbandale Coal Mine, Des Moines, Iowa. (see p. 323.)

Horizon: Pennsylvanian, Des Moines Series.

Type specimen: No. WCB429, Henry Shaw School of Botany, paleobotanical collections.

This species is named in honor of Charles Shuler Bendixen, an official of the Shuler Coal Mine, located eight miles west of Des Moines, Iowa.

Schopfiastrum decussatum, gen. et sp. nov.

The following description is based on two stem fragments also found in coal balls from the Urbandale mine near Des Moines. Although much remains to be learned of the plant as a whole the available information indicates a pteridosperm with strikingly distinct anatomical features, particularly the structure and arrangement of the leaf traces. It is intended that this paper serve only as a preliminary report to be supplemented by a full account when more complete specimens are forthcoming. Of the two specimens at hand, one (WCB434) consists of a decorticated stele, and another (WCB421) in which both stele and outer cortex are preserved.

Judging from the more complete specimen (fig. 17) the stem was about 21 mm. in diameter. The specimen illustrated (measuring 9. x 30. mm.) appears collapsed due to decay of the internal cortical tissues. However, there is no reason to believe that the stem was not cylindrical or nearly so in life.

The primary wood is rather sharply 4-angled in transverse section, measuring 2. mm. on a side, and is composed of elongate tracheids with some interspersed parenchyma. The infiltrations of pyrite, as well as occasional cracks (fig. 18), have destroyed a few of the cells. The parenchyma cells were relatively few, far

less than in pteridosperm stems (presumably the most closely related to this genus) such as *Rhetinangium* and *Heterangium*.

In both their structure and order of departure from the stele the leaf traces differ in striking fashion from any other stem that has been referred to the Pteridospermeae. In the stem shown in fig. 17 it may be noted that, although the primary xylary tissues are crushed, the two leaf traces appear to be diametrically disposed, that is, 2-ranked. This opposite arrangement is confirmed in the other specimen (WCB434) in which the primary wood is somewhat better preserved (fig. 18).

At the earliest observed point in its departure from the stele the trace is already bilobed (fig. 18, lt_2) and its cross-sectional size is equal to fully half that of the cauline primary xylem. Even at this level, however, each lobe has in turn started to divide, there being four exarch protoxylem groups in the trace. Reason for believing that these four originated from two exarch groups in the primary stele will be given below. In following this leaf trace through the length of the specimen (3 cm.) very little change takes place, but the opposite trace, as well as the one shown in fig. 17 (from specimen 421), reveals at least some of the subsequent changes. The trace as a whole becomes strongly 4-lobed and tangentially elongate with the protoxylems occupying the outermost tip of each lobe. Shortly prior to its departure from the stele the protoxylem groups in the distal lobes of the trace divide (fig. 18, pxt). It is not known whether further lobing or actual division occurs in the traces at a higher level.

Judging from the difference in the structure of a pair of traces at any one point, it is evident that one departed appreciably in advance of the other. The origin of the succeeding pair of traces may next occupy our attention. In two of the ground sections prepared from specimen 434, four exarch protoxylem groups are clearly defined in the stele. These are paired, and alternate with the leaf traces, as shown in fig. 18, pxs. It seems most probable that the next pair of traces will have their origin from these protoxylem points, although confirmation of this must await the discovery of longer stem fragments.

Judging from these opposite pairs of protoxylem groups, an individual trace started with but 2. Shortly following segregation of the trace from the primary stele of the stem each protoxylem divided, resulting in 4, and this was then followed by the lobing of the traces as a whole into 4 parts.

As the traces increased in their tangential dimensions in passing through the secondary wood they left a broad gap in that tissue and a small quantity of secondary wood accompanied the trace on its outer face (fig. 22, x 2).

The secondary xylem of the stem is strongly developed, attaining a maximum radius of nearly 3 mm. The shape of the tracheids as seen in transverse section is of the irregular angular type found in other pteridosperm stems (Andrews, '40, p. 89) such as *Lyginopteris* and *Medullosa* (Anglorota section), and their radial walls are covered with closely compacted angular bordered pits. The same type of pitting is also found in the metaxylem tracheids.

The outer cortex is constructed of thin longitudinal-radial plates of fibers

(fig. 20) rather regularly spaced with parenchymatous cells. Although the latter vary appreciably in size most of them are characteristically elongated (140. x 80. μ) in a tangential direction. The fibrous strands anastomose only at extended intervals, appearing in tangential section (fig. 21) as nearly parallel strands. This fibrous zone of the cortex is separated from the epidermis, the cells of which measure 35. x 20. μ, by a single row of cortical parenchyma cells considerably smaller than those deeper within.

Diagnostic Summary of the Genus Schopfiastrum.—

Outer cortex composed of nearly parallel radial-longitudinal fibrous strands separated by tangentially elongated parenchyma cells; secretory canals absent or very rare; primary xylem a mixed protostele, protoxylem exarch, two opposite pairs present in internodes; leaf traces large, soon becoming bilobed and accompanied at least to the inner cortex by a small amount of secondary wood; traces arranged in opposite pairs, one of each pair departing ahead of the other, the succeeding pair of traces alternating; secondary wood present, with closely compacted, reticulate-bordered pits on radial walls of tracheids.

Locality: Urbandale Coal Mine, Des Moines, Iowa.

Horizon: Pennsylvanian, Des Moines Series.

Type specimens: Nos. WCB421 and WCB434, Henry Shaw School of Botany, paleobotanical collections.

The genus is named for Dr. James M. Schopf, of the United States Bureau of Mines, in recognition of his contributions to Carboniferous paleobotany.

Discussion.—

The genus *Schopfiastrum* is considered referable to the Pteridospermeae in view of the characteristic fibrous outer cortex, the structure of the leaf traces which compares in a general way with that of *Rhetinangium* and *Heterangium*, and the closely compacted reticulate pitting of the tracheids as well as their shape as observed in transverse section.

The closest affinities of *Schopfiastrum* seem to lie with the genera *Rhetinangium* and *Heterangium*. In fact, during the earlier phases of this investigation the stem was tentatively considered as a new species of *Rhetinangium*. However, when the structure of the primary xylem and the organization of the traces had been worked out this treatment was found to be quite untenable. The outer cortex is very similar to that of *Rhetinangium arberi* Gordon ('12), both possessing the distinctive tangentially elongated parenchyma cells between the fibrous strands. However, *Schopfiastrum* lacks the secretory canals, with their radially arranged epithelial parenchyma, which are present in the outer cortex of *Rhetinangium*. A few apparent canals have been observed in this region but in almost all cases they are clearly the result of fungous or bacterial action. The leaf traces of the Iowa stem are comparable with those of *R. arberi*, in their massive size and exarch protoxylems but the comparison goes no further. In *Rhetinangium* the trace is composed of a number of irregularly U-shaped strands interspersed with

parenchyma, quite different from the predominantly tracheidal unit traces of *Schopfiastrum*.

There is no evidence in the Iowa stem of horizontal sclerotic plates in the inner cortex such as are found in *Heterangium*. The single leaf trace (to each petiole) of *Schopfiastrum* precludes any close relationship with the polydesmic Heterangiums and differs markedly in size, form, and arrangement from any of the Eu-heterangium species.

Schopfiastrum is of special interest not only in its clearly defined generic characters, but, judging from the structure of the primary wood, it is a rather primitive pteridosperm. As stated above, it seems to lie as close to *R. arberi* as any described pteridosperm yet the protostele of *Schopfiastrum*, its less complex leaf traces, and the lack of secretory canals in the cortex, all point toward a less specialized plant. Also, the monotypic *Rhetinangium* was found in the middle Lower Carboniferous of Scotland while *Schopfiastrum* is of much more recent origin, coming from middle Upper Carboniferous rocks. This is an apparent paradox that is by no means unique in paleobotany and at least offers assurance that there are many more unknown species and genera in the Seed-fern group that remain to be found.

Appendix 1.

The late Professor A. C. Noé is quite justly acknowledged as having been the first person to collect coal balls in this country, nearly 25 years ago, and to him is due most of the credit for initiating interest in their study. However, Mr. F. O. Thompson has recently brought to my attention a point of historical interest pertaining to an earlier discovery of coal balls in America that seems worth mentioning.

In Volume II of the Iowa Geological Survey Reports Dr. C. R. Keyes ('94) reported the presence of coal balls (although they were not described under that name) in the Bloomfield mine near Des Moines. To quote directly from this account:

> Associated with the coal bed are numerous lenticular masses of ironstone. They vary in size from a few inches to ten or twelve feet horizontally, and up to six feet vertically. The smaller ones are commonly called "nigger heads." These segregations occur in all parts of the coal seams, but they are most abundant in the roof and upper surface of the coal, where they are often so close together as to form an almost continuous layer. It is common for the nodules in the upper part of the coal to project upwards above the top of the seam, often two to three feet in case of the large masses. They are covered usually by a few inches of carbonaceous material. [pp. 279-281].

In the Annual Report for 1896 A. F. Bain ('97) reported the presence of "clay-ironstones" in the Bloomfield mine and several others, and he notes that: "An analysis of similar material from a mine in Mahaska County showed the presence of about 88 per cent of limestone, with 8 per cent of organic matter and small percentages of iron oxides and sulphides." (p. 297)

The authors in both instances figure vertical sections through the coal showing the coal balls ("ironstones") in the upper portion of the seams. Thus there is no

possibility of their being confused with the sterile slate-gray concretions common in the overlying shales, and also referred to by the miners as "nigger heads." The presence of 8 per cent organic matter removes any doubt as to their identity, and the high percentage of limestone reported by Bain also suggests coal balls of fine quality. At least his figures leave little room for the obnoxious iron sulphide.

These petrifactions, although apparently well known to geologists, did not reach the hands of paleobotanists until a quarter of a century later when Noé started collecting in Illinois.

Appendix 2.

Medullosa anglica var. *thiesseni* was described by Schopf in 1939 although at that time its origin was unknown beyond the knowledge that it had been collected in the "western coal fields of this country." The horizon from which var. *thiesseni* came has now been established and the information is presented here, with Dr. Schopf's permission, in order that the record of the western Medullosas may be as complete as possible.

The specimens upon which Schopf's ('39) description were based were found by R. V. Pepperberg in southeastern Nebraska in 1907. Three years later Pepperberg described a Carboniferous flora, in which *Neuropteris* predominated, from two localities, one near Nebraska City (Otoe Co.) and the other near Peru (Nemaha Co.). It is not clear at which locality the specimens were found but the horizon is believed to be the same. The specimens are mentioned on p. 330 (Pepperberg, '10) in a letter addressed to him by David White. The generic identity of the specimens was established by Reinhardt Thiessen, for whom they were later named.

In 1936 Pepperberg's collections were referred to by Elias ('36) as follows:

> 19. Near the top of the Table Creek shale formation. The well-preserved flora, collected in soft sandy shale 1½ feet below the Dover limestone, contains *Neuropteris* and *Annularia.* Probably from this horizon came the flora consisting of numerous *Neuropteris* leaves and *Calamites* stems in sandstone and sandy shales at Brownville, Nemaha County, Nebraska . . . and at Nebraska City, Otoe County, Nebraska (7, p. 313).[6]

In 1943 Condra and Reed reported that "the so-called 'Table Creek shale' represents three formations, i. e., the shale below the Dover (with the Maple Hill limestone missing), plus the sandstone equivalent of the Tarkio, plus the Willard shale." In the stratigraphic column given by Condra and Reed (p. 42) the "Table Creek" is from the lower part of the Richardson sub-group. The latter is the upper sub-group of the Wabaunsee group which is the uppermost part of the Virgil series.

It is of considerable interest to find a *Medullosa* of the Anglorota group from such a high horizon. All of the English species were derived from the Lower Coal Measures, well down in the lower part of the Pennsylvanian. The American *M. distelica* and *M. Thompsonii* likewise are from horizons considerably below the Table Creek shale. Thus it is evident that the Medullosas of the *anglica* type were not only widely distributed geographically but that this racial life span extended over many millions of years.

[6] This is a reference to Pepperberg's 1910 paper.

Acknowledgment.—

The addition of this chapter to American coal ball studies has been made possible through a gift from Mr. Frederick O. Thompson of selected specimens from his own collections. For his generous support and continued interest in the scientific progress of the investigation we are profoundly grateful. Thanks are also due Mr. R. M. Kosanke for the loan of slides of *Medullosa anglica* var. *thiesseni* from the collections of the Illinois Geological Survey; and to Mr. F. Tracy Hubbard for the loan of Iowa coal ball slides from the Botanical Museum of Harvard University. I am indebted to Dr. James M. Schopf, The United States Bureau of Mines, for many helpful suggestions pertaining to the taxonomy of *Medullosa*.

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Explanation of Plate

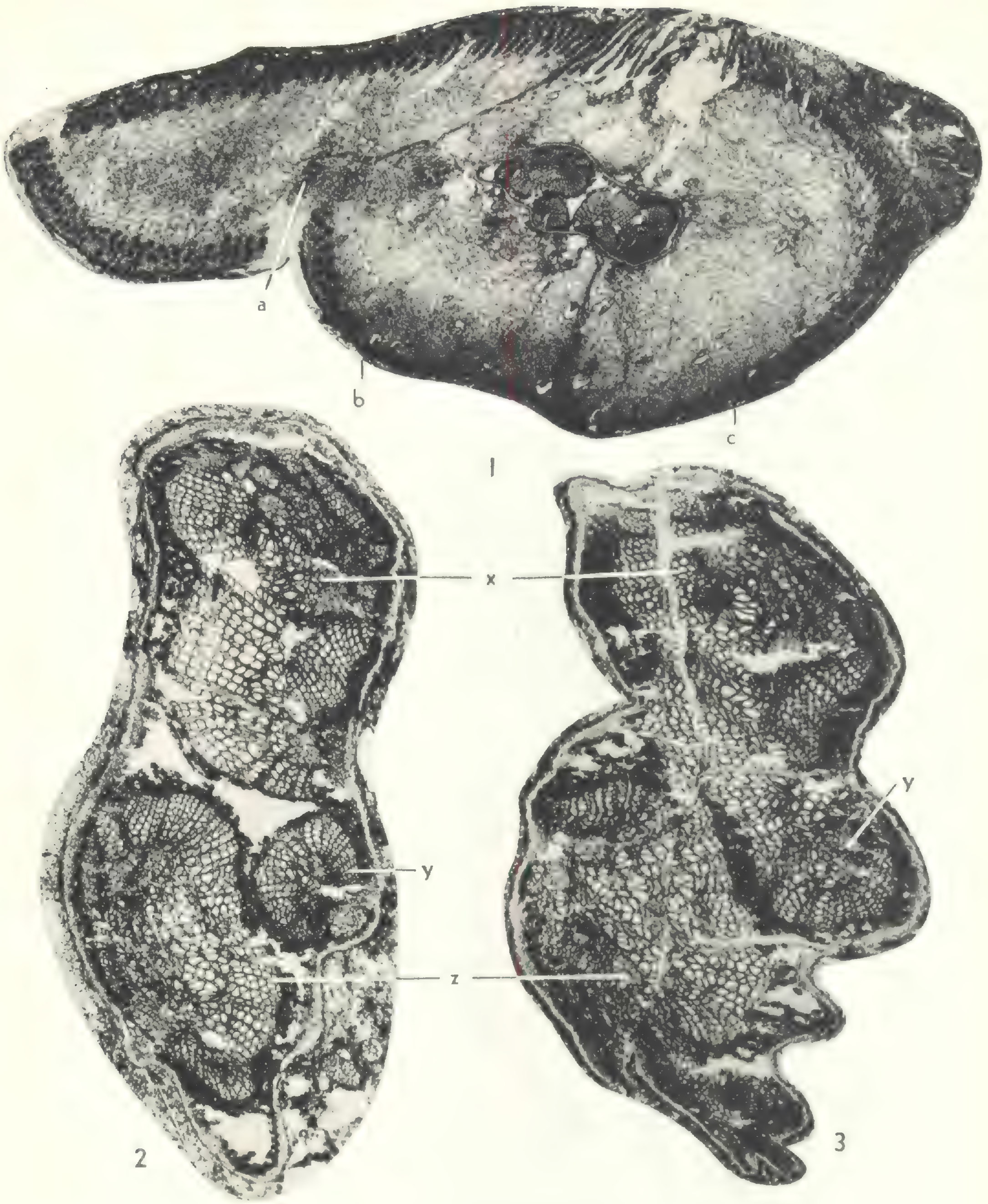
PLATE 5

Medullosa Thompsonii Andrews

Fig. 1. The stem in transverse section showing a leaf trace departing at the left. *a*, fibrous strands separating stem from leaf base; *b*, *c*, between these two points the cortical rind is lacking, explanation in text. WCB420-2-A3, x 2.3.

Fig. 2. Stelar system from fig. 1 shown at a higher magnification. *x*, *y*, *z*, steles. WCB420-2-A3, x 7.0.

Fig. 3. Showing fusion of the three steles. WCB420-1-A3, x 7.0.



ANDREWS—AMERICAN CARBONIFEROUS FLORAS. VII

EXPLANATION OF PLATE

PLATE 6

Medullosa Thompsonii Andrews

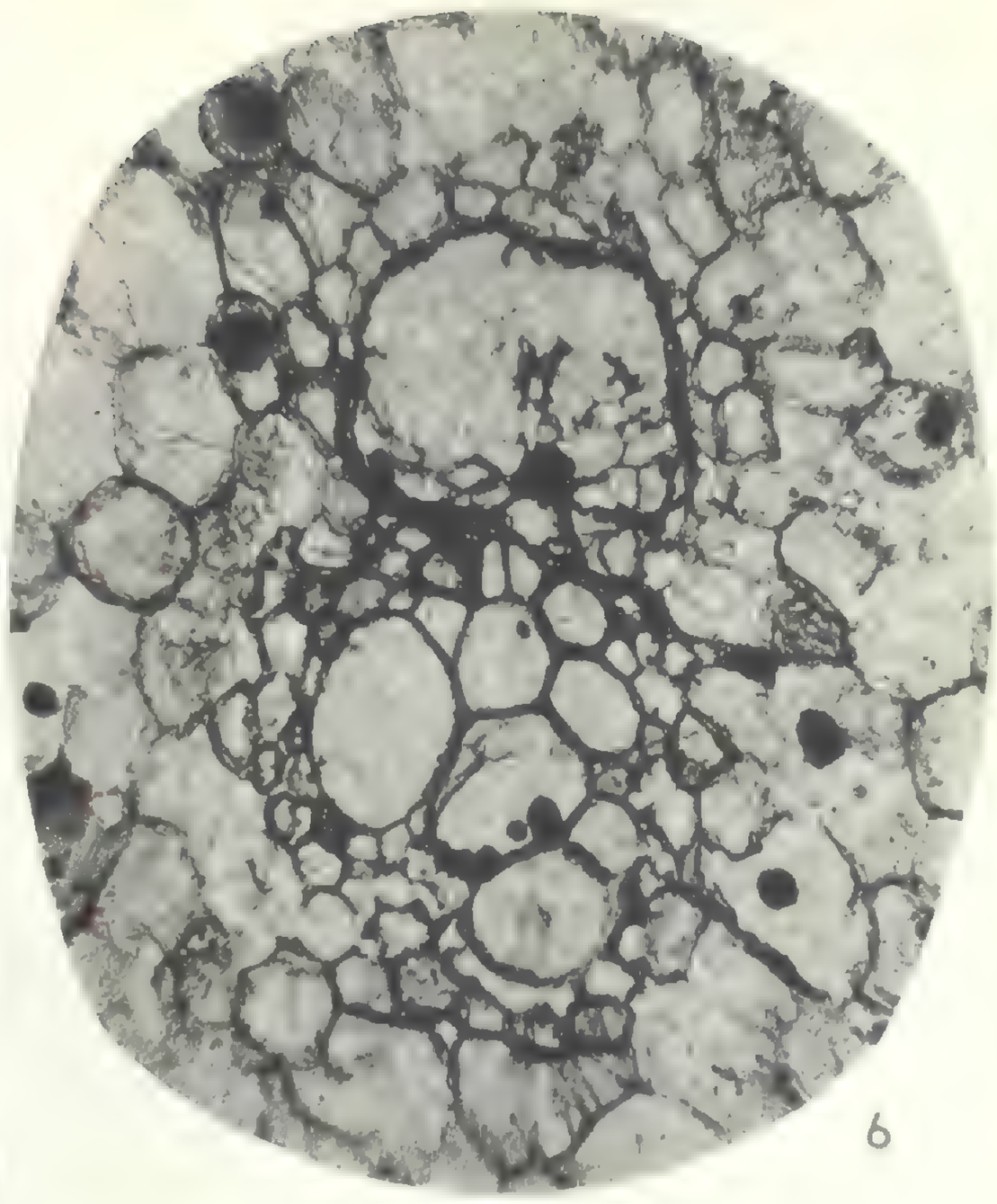
Fig. 4. Transverse section through outer cortex as that tissue appears between points *b* and *c* of fig. 1. Slide No. 1363, x 17.5.

Fig. 5. The rind or outer cortical fibrous tissue of the stem. Slide No. 1364, x 17.5.

Fig. 6. A single vascular bundle from the petiole shown in fig. 9. WCB426, Slide No. 1368, x 76.

Fig. 7. *Medullosa* sp. A bistelar Medullosan stem. Explanation in text. WCB461, x 4.2.

4



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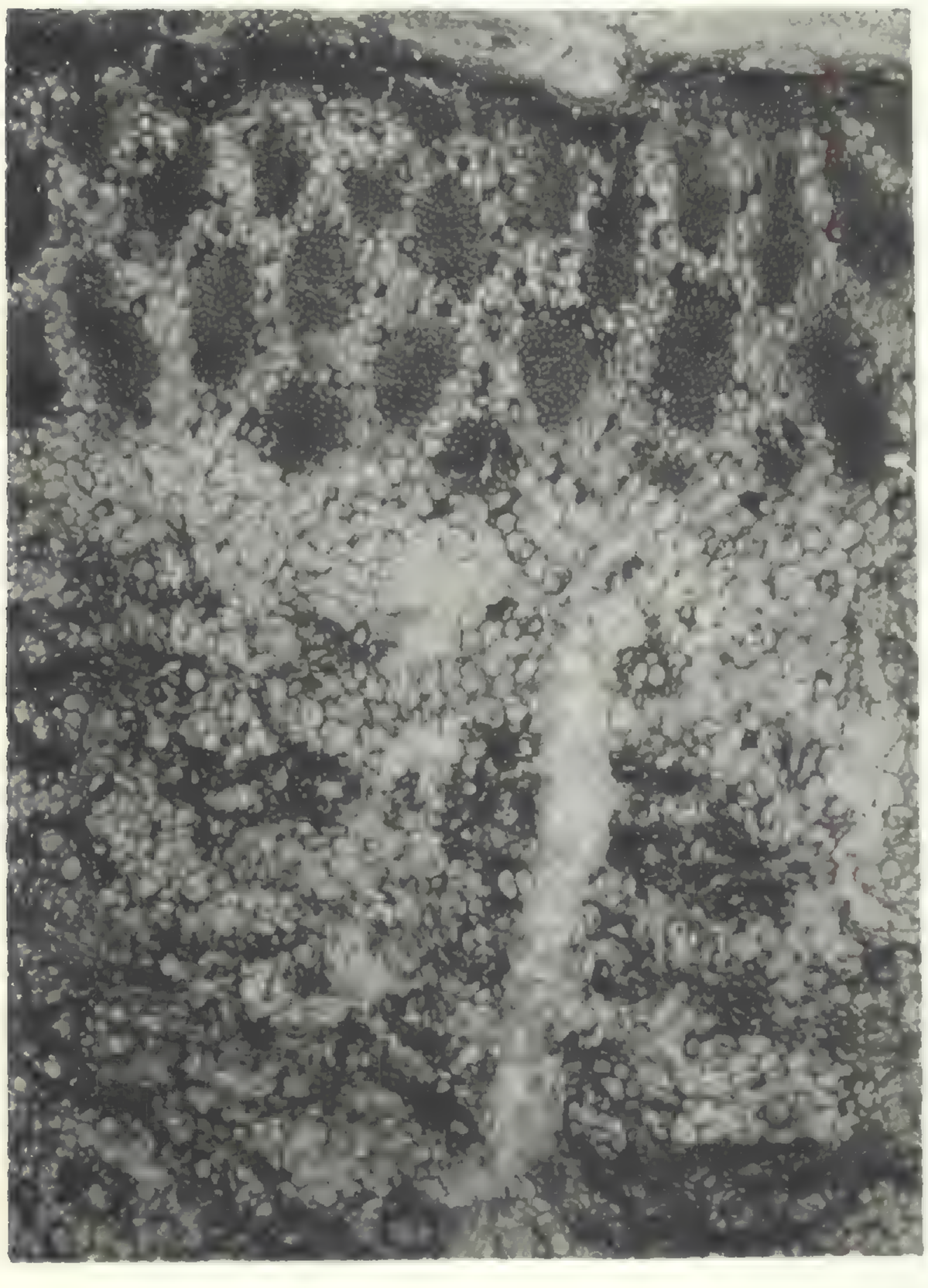

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7

ANDREWS—AMERICAN CARBONIFEROUS FLORAS. VII

Explanation of Plate

PLATE 7

Myeloxylon Bendixenii Andrews

Fig. 8. Transverse section showing the greater part of the petiole. WCB429-S2, x 10.2.

ANDREWS—AMERICAN CARBONIFEROUS FLORAS. VII

EXPLANATION OF PLATE

PLATE 8

Medullosa Thompsonii Andrews

Fig. 9. An isolated petiole believed to be referable to the stem species shown in fig. 1. WCB426-T2, x 10.2.

ANDREWS—AMERICAN CARBONIFEROUS FLORAS. VII

EXPLANATION OF PLATE

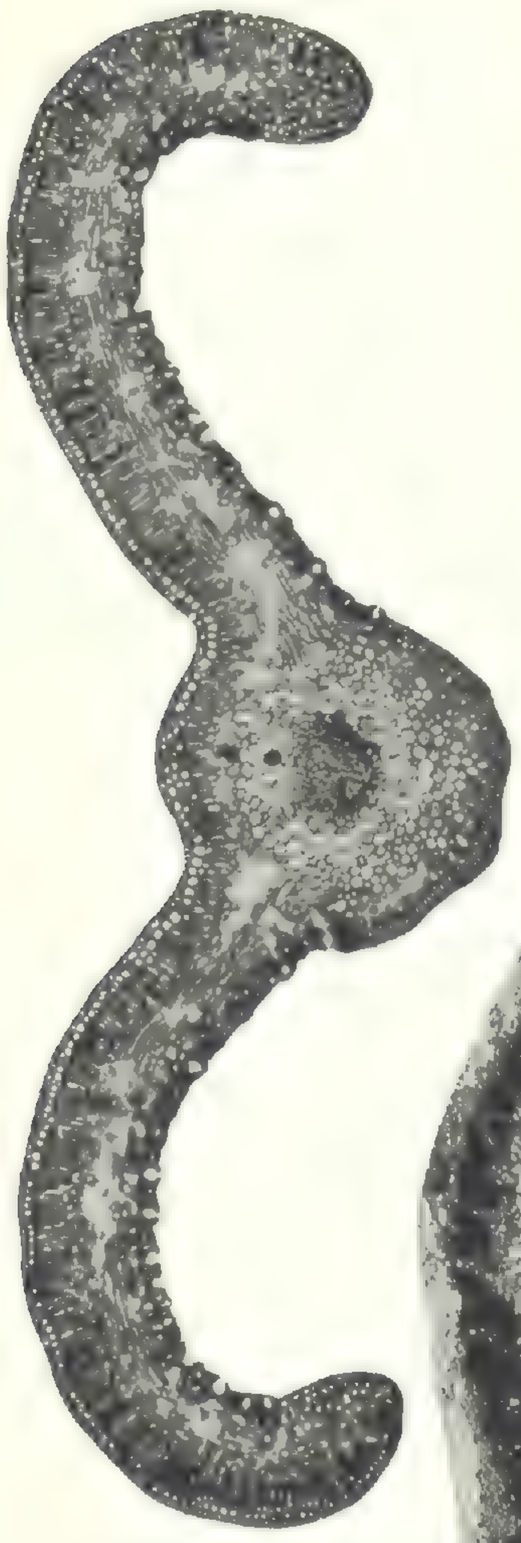
PLATE 9

Fig. 10. Transverse section of a leaflet of the living *Cycas revoluta*, x 20.2.

Fig. 11. Showing a large multicellular hair, and basal portion of another, from the lower epidermis of an *Alethopteris*-like leaflet. Slide No. 1369, x 99.

Figs. 12, 13. Transverse sections of rachis branches believed to be referable to *Medullosa Thompsonii:* fig. 12, WCB433-T1, x 11; fig. 13, WCB429-S2, x 10.2.

Fig. 14. A portion of the outer stelar region of *Medullosa Thompsonii*, showing departing leaf traces. WCB420-2-B3, x 22: *a*, leaf trace shortly after departure from stele; *b*, leaf trace dividing.



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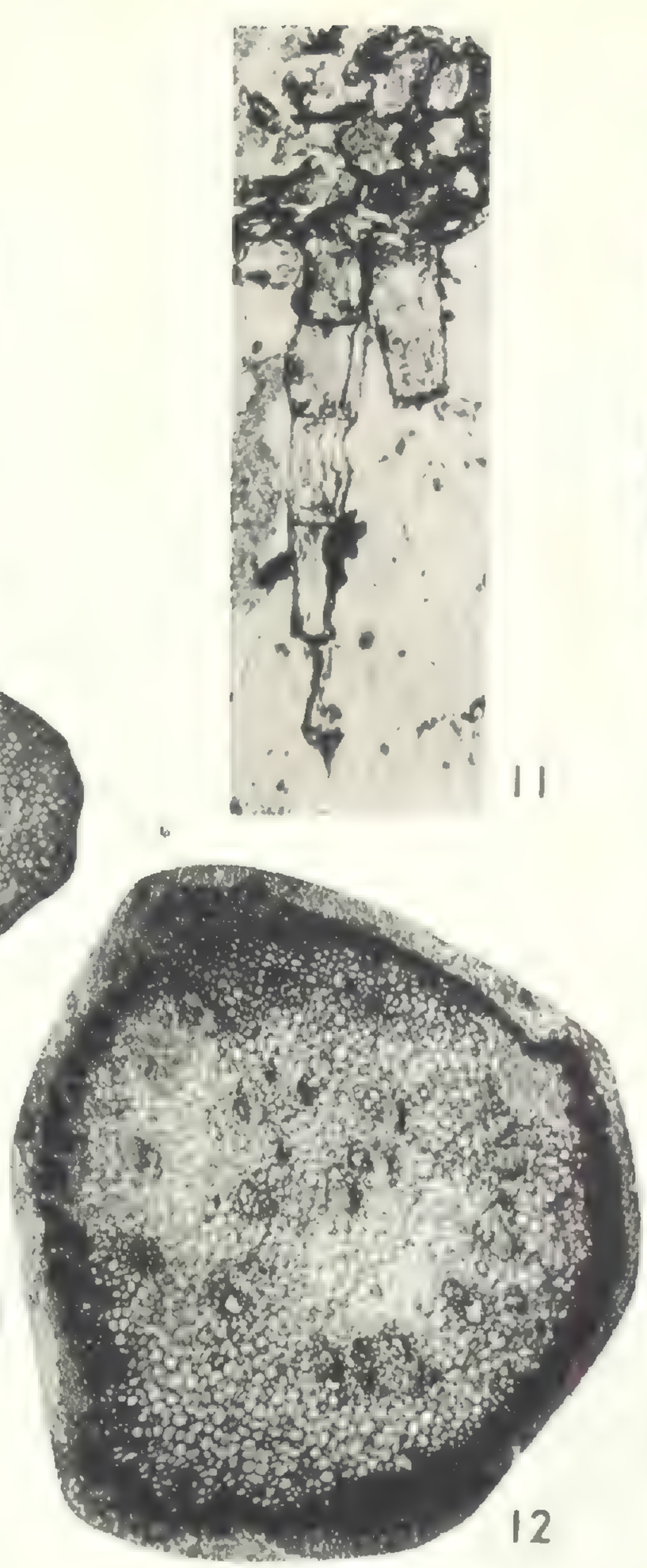

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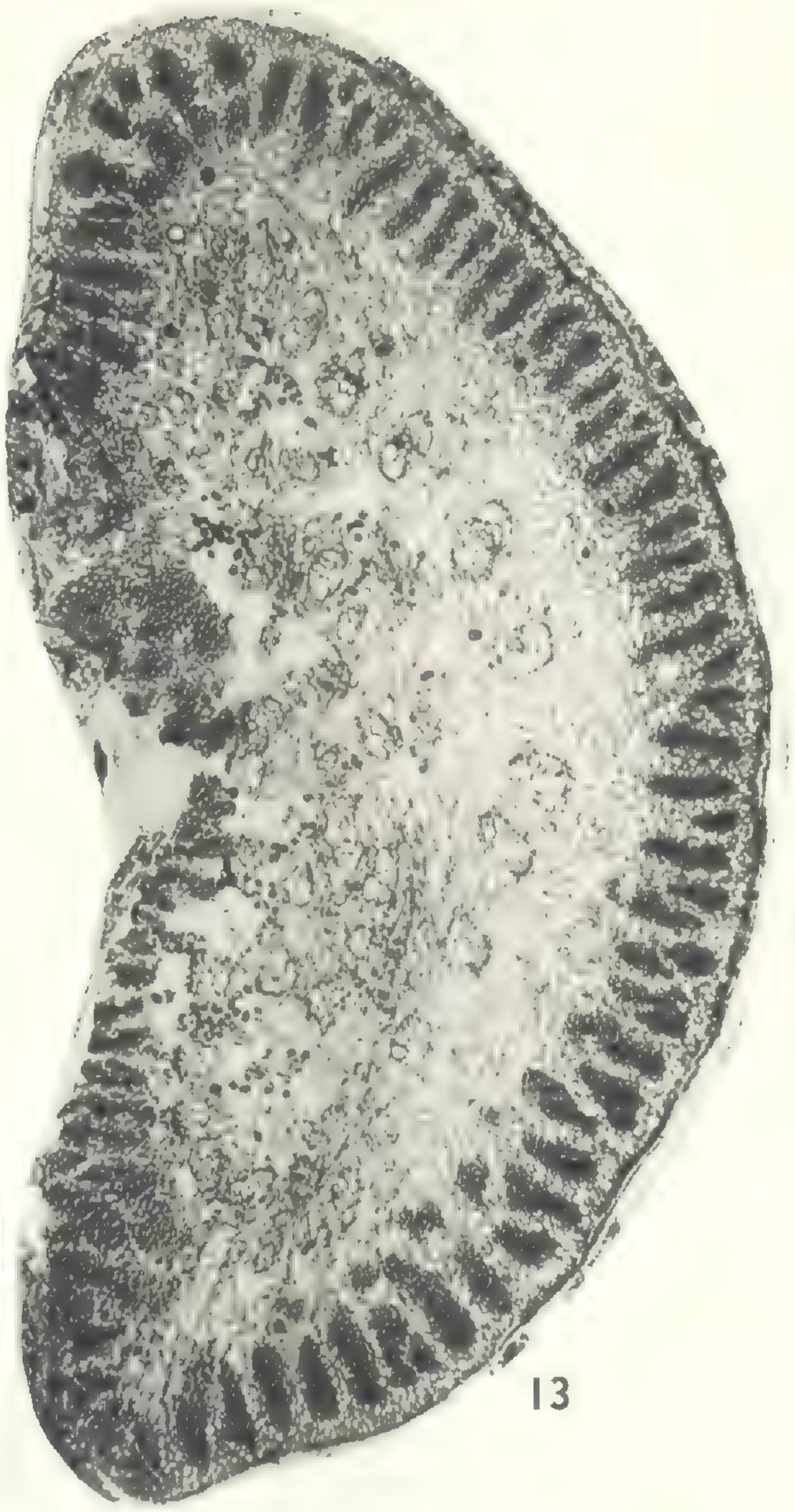

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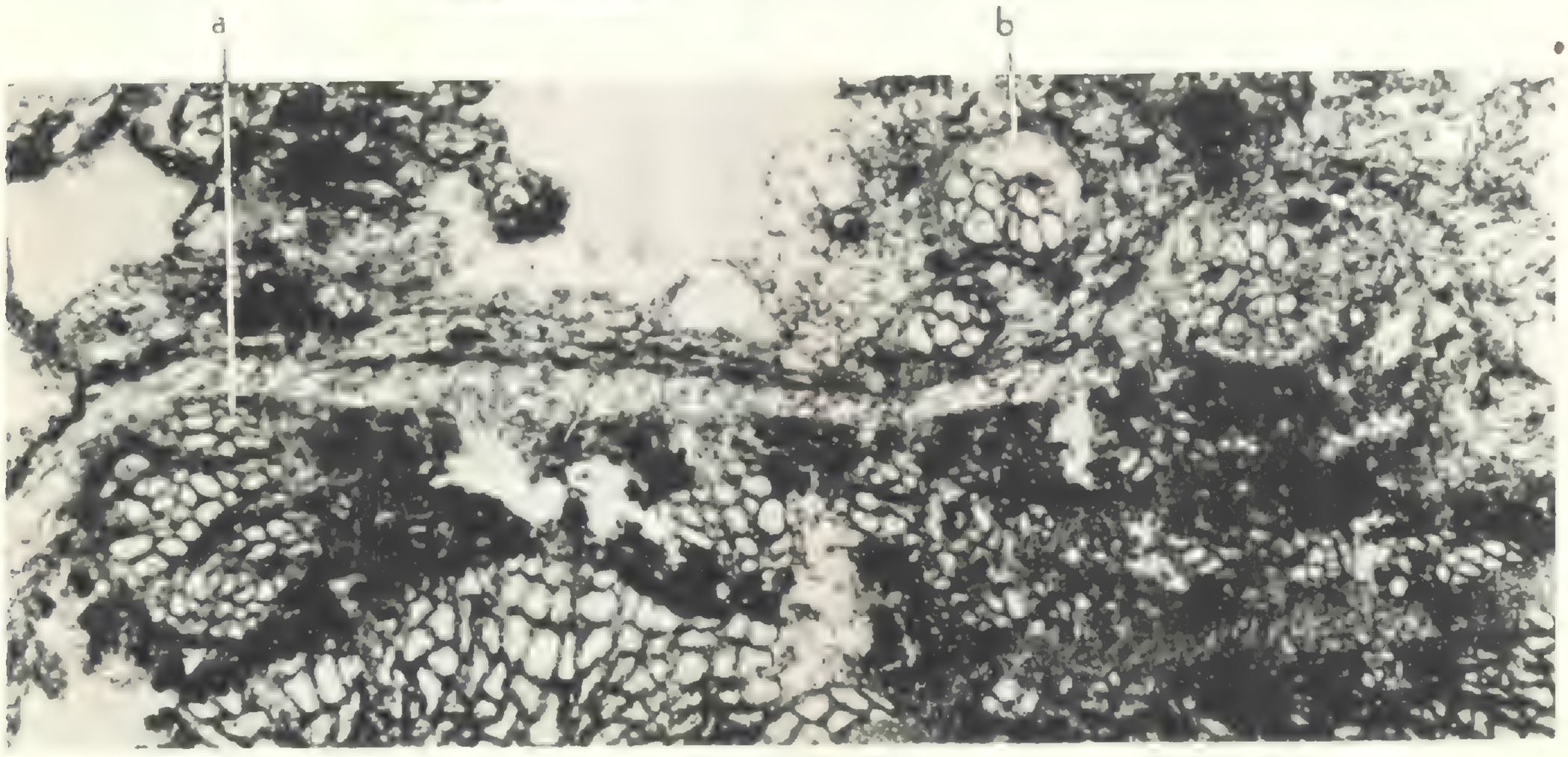


14

ANDREWS—AMERICAN CARBONIFEROUS FLORAS. VII

Explanation of Plate

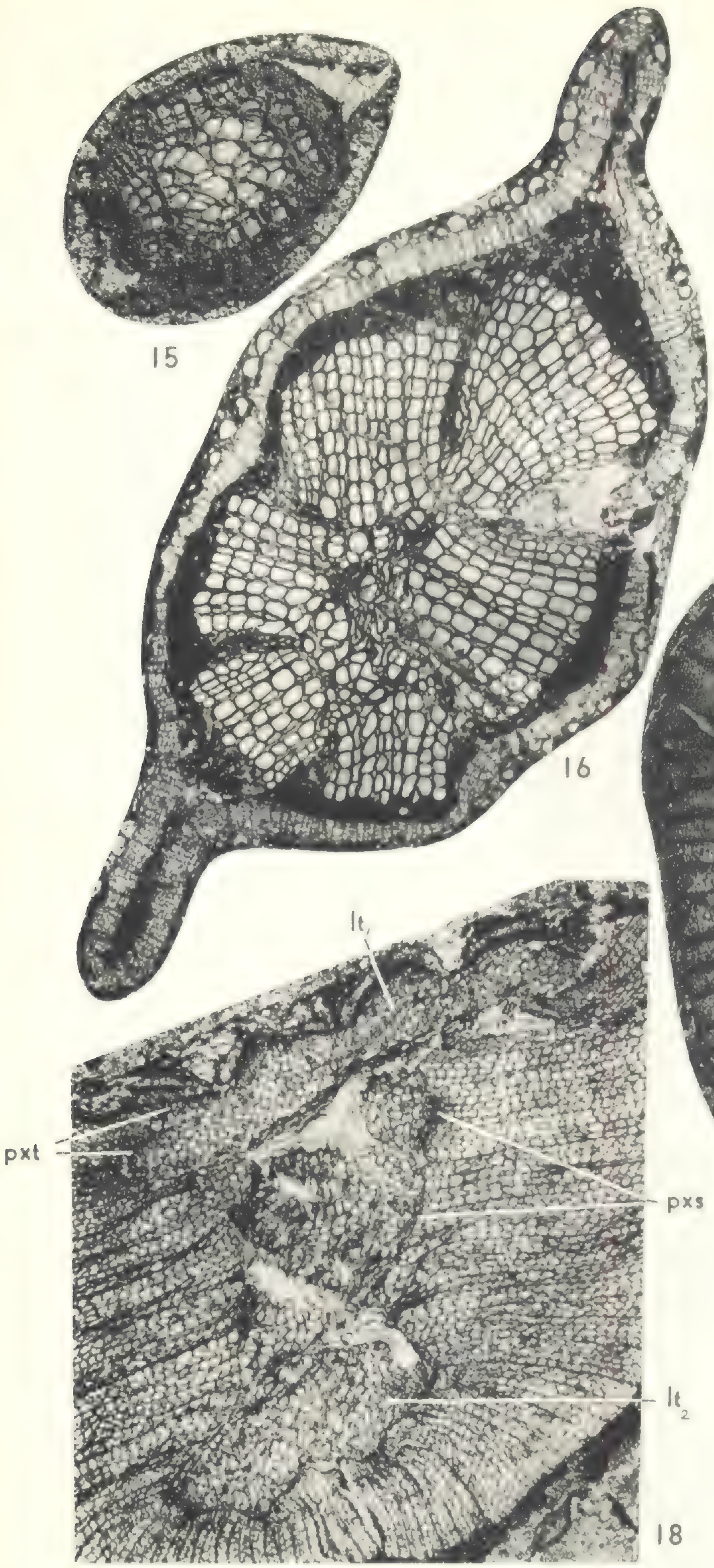
PLATE 10

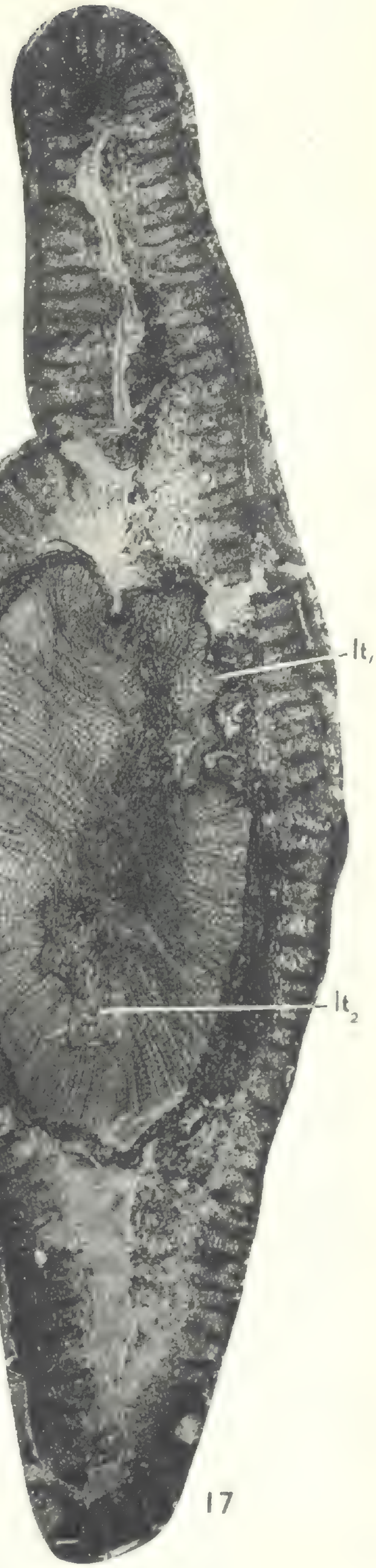
Figs. 15, 16. Roots found in coal balls associated with the Medullosan stems: fig. 15. WCB462, x 14; fig. 16. WCB422, x 14.

Schopfiastrum decussatum Andrews

Fig. 17. Entire stem in transverse section: lt_1 and lt_2, leaf traces. WCB421, x 6.5.

Fig. 18. Showing central portion of stem (from another specimen) at a higher magnification: lt_1, lt_2, leaf traces; pxs, protoxylem groups of stele; pxt, protoxylem groups of trace. WCB434. Slide No. 1353, x 11.





ANDREWS—AMERICAN CARBONIFEROUS FLORAS. VII

Explanation of Plate

PLATE 11

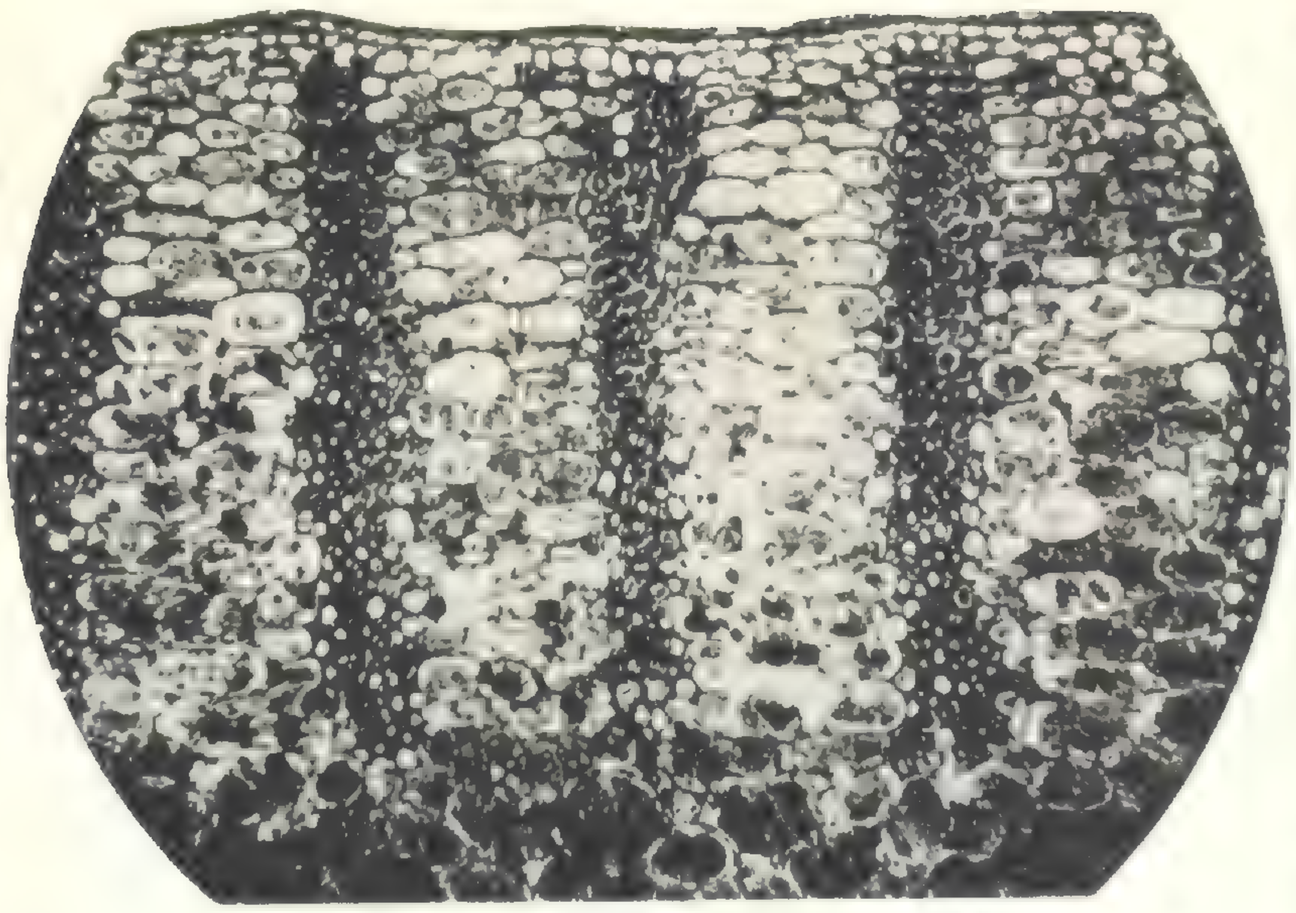
Fig. 19. Transverse of rachis branch believed to be referable to *Medullosa Thompsonii*. WCB427, x 10.2.

Schopfiastrum decussatum Andrews

Fig. 20. The outer cortex in transverse section. Slide 1359, x 37.5.

Fig. 21. The outer cortex in tangential section. WCB421-B-S4, x 8.5.

Fig. 22. Leaf trace departing from stele. This is a higher magnification of lt_1 in fig. 17; x 18.5.



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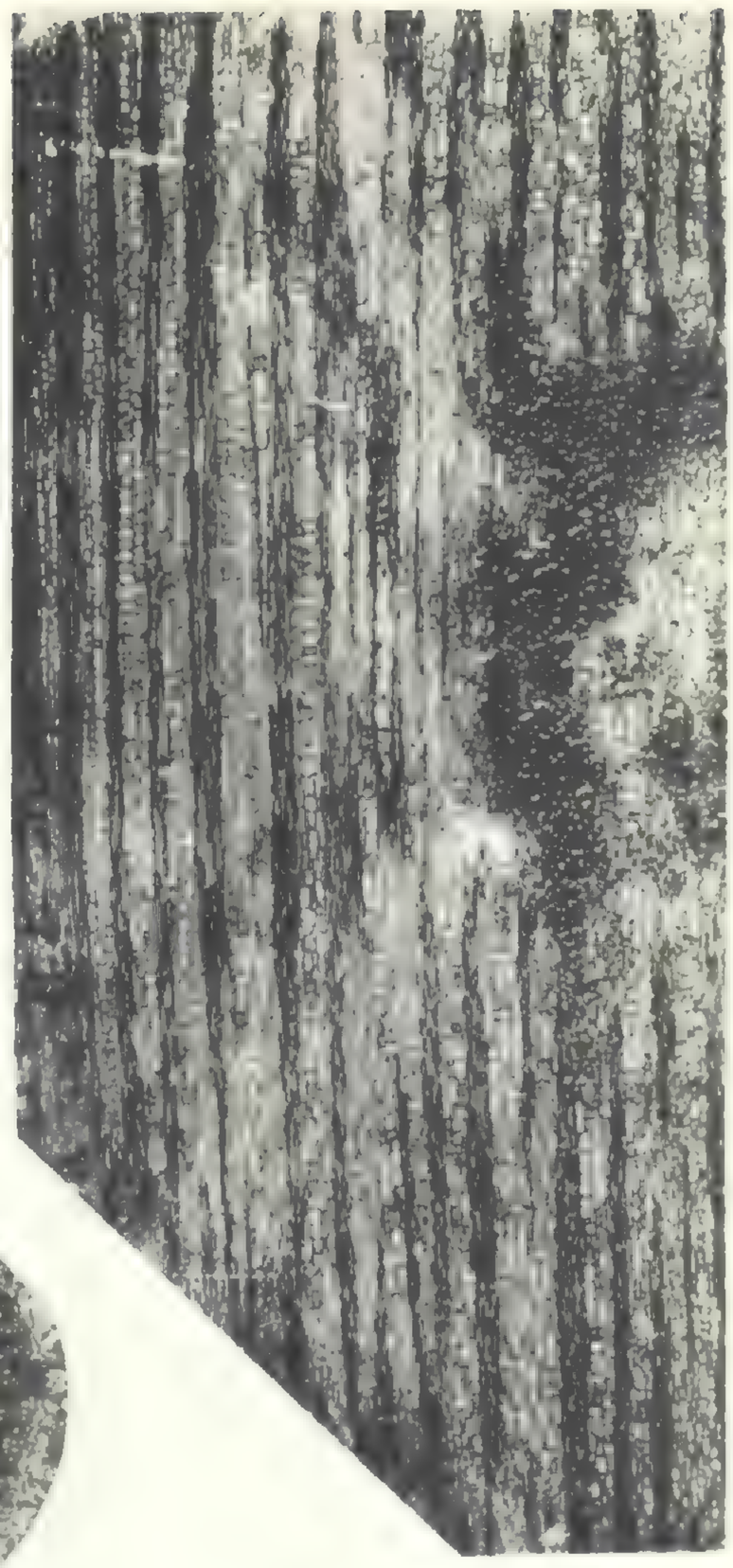

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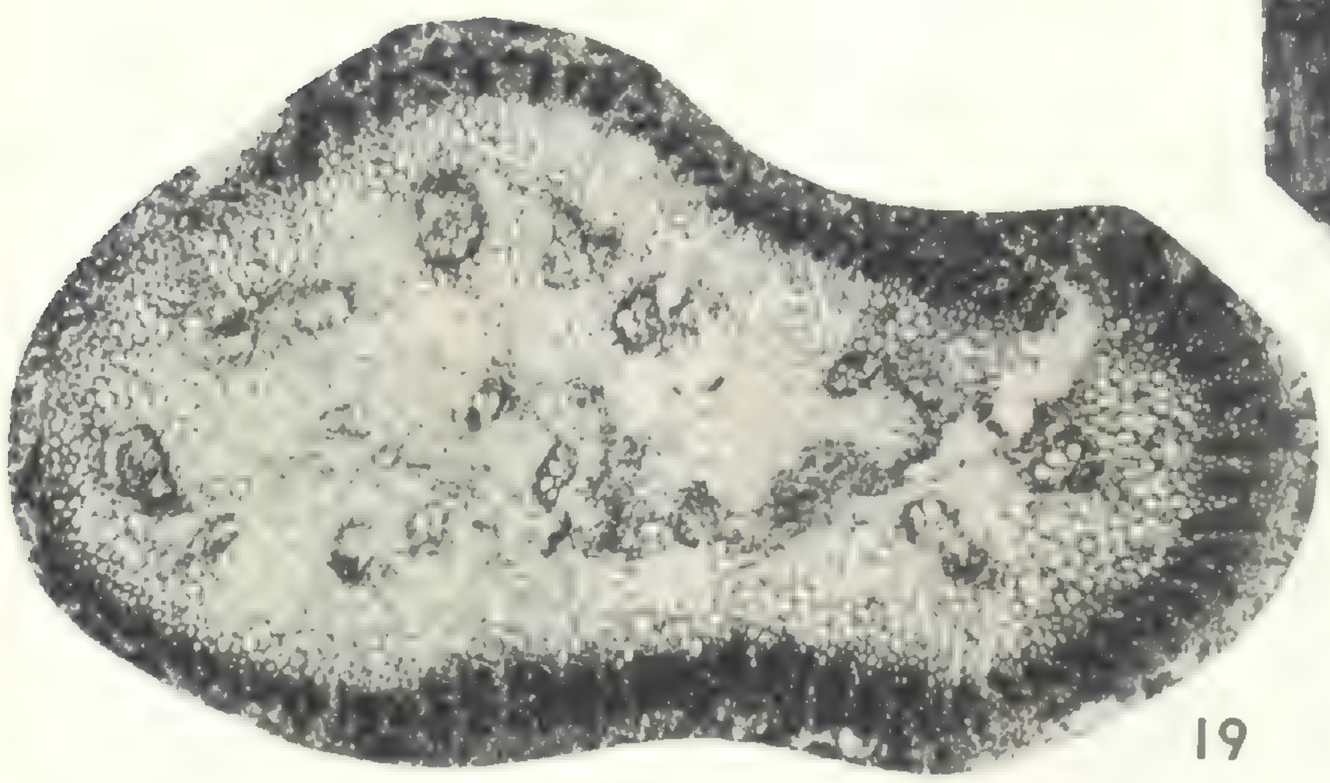

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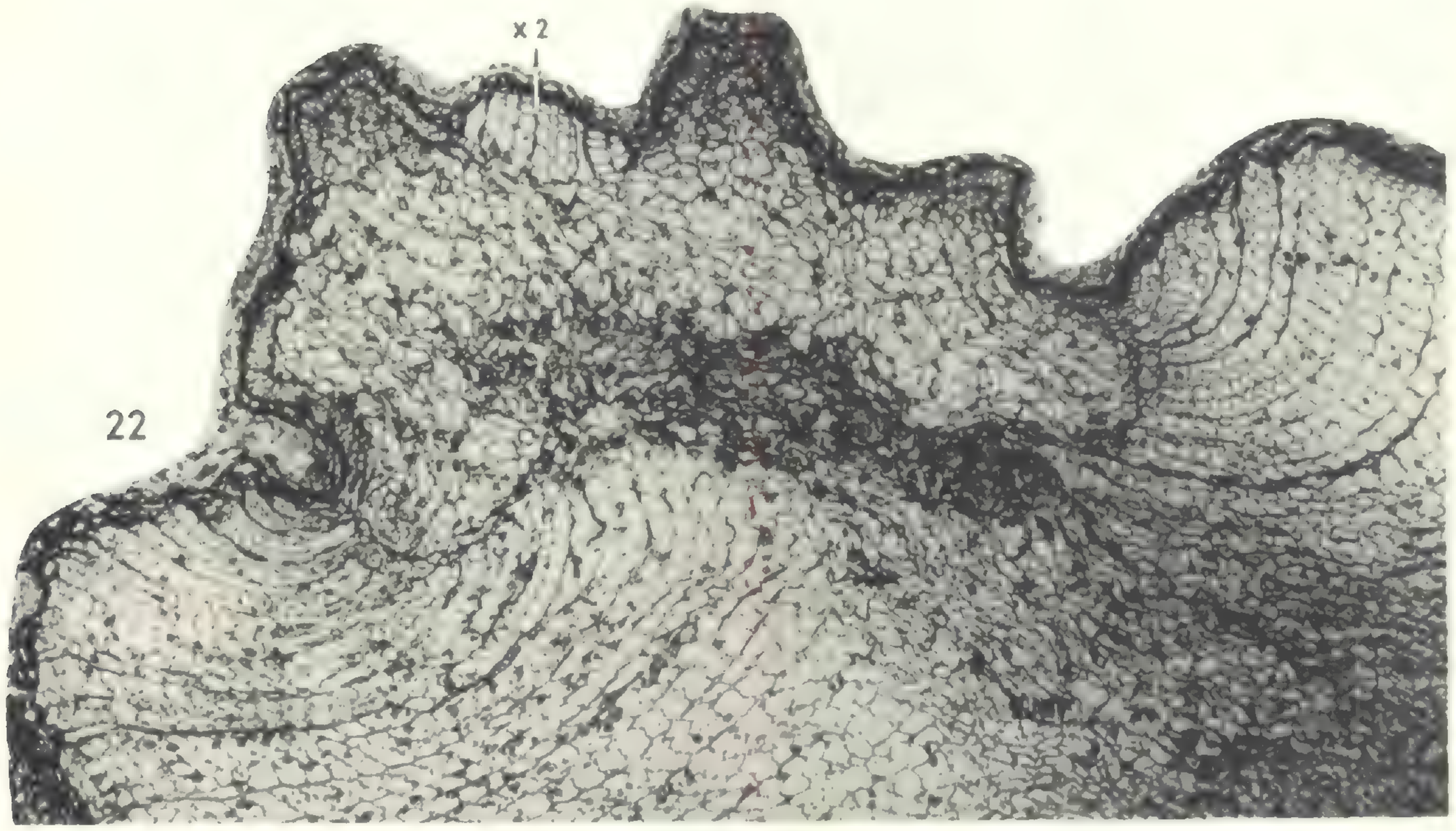


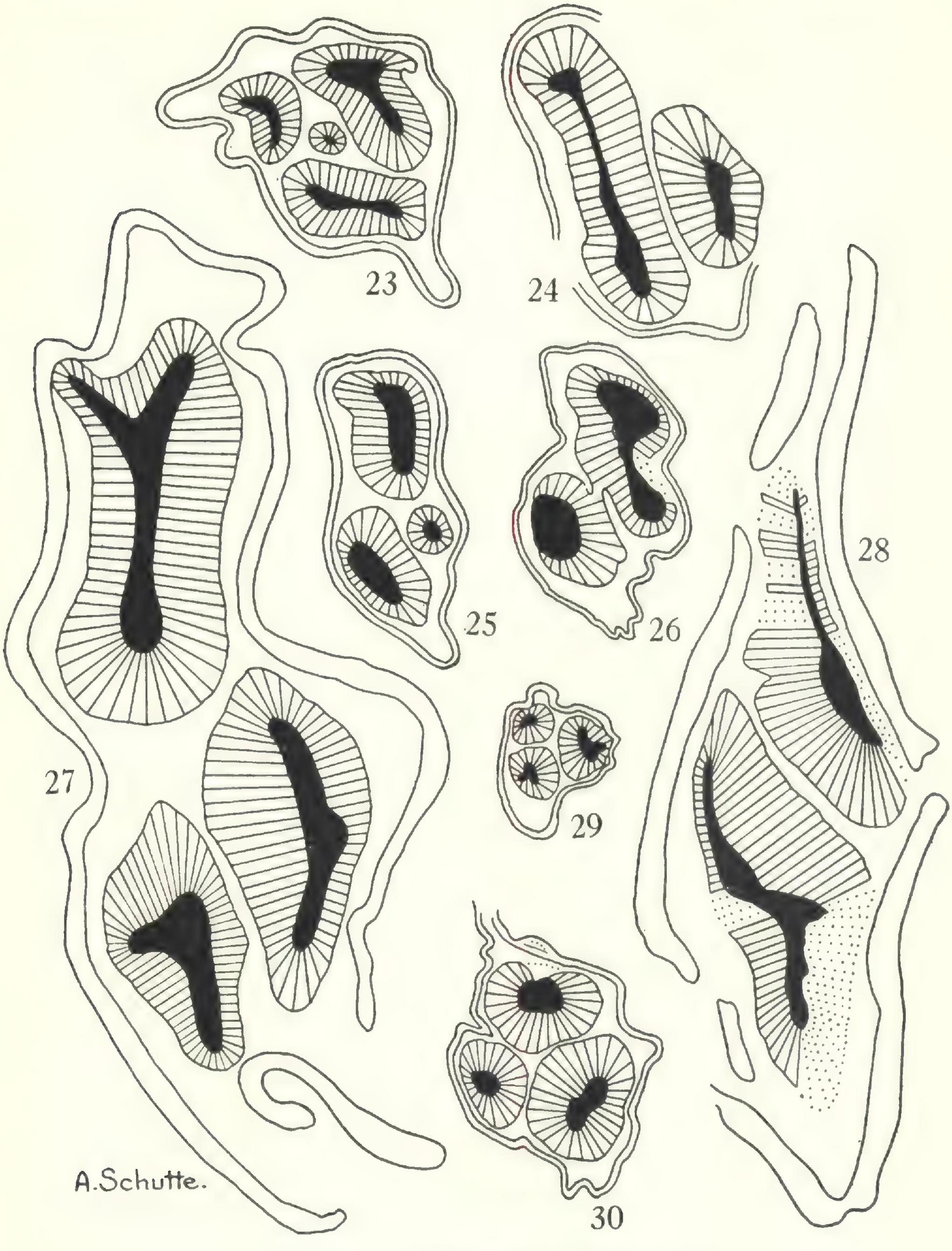
22

ANDREWS—AMERICAN CARBONIFEROUS FLORAS. VII

Explanation of Plate

PLATE 12

Diagrams of the periderm and stelar systems only of the closely related species of *Medullosa* referable to the sub-genus Anglorota. The periderm is indicated by the enclosing, or partially enclosing, double line, the primary xylem in solid black, and the secondary xylem by radiating lines. *The magnification is x 2.8 in all cases.* Fig. 23. M. *centrofilis* De Fraine; fig. 24. M. *sp.* (see page 335); figs. 25, 26. M. *Thompsonii* Andrews; fig. 27. M. *anglica* Scott; fig. 28. M. *distelica* Schopf; fig. 29. M. *pusilla* Scott; fig. 30. M. *anglica* var. *thiesseni* Schopf.

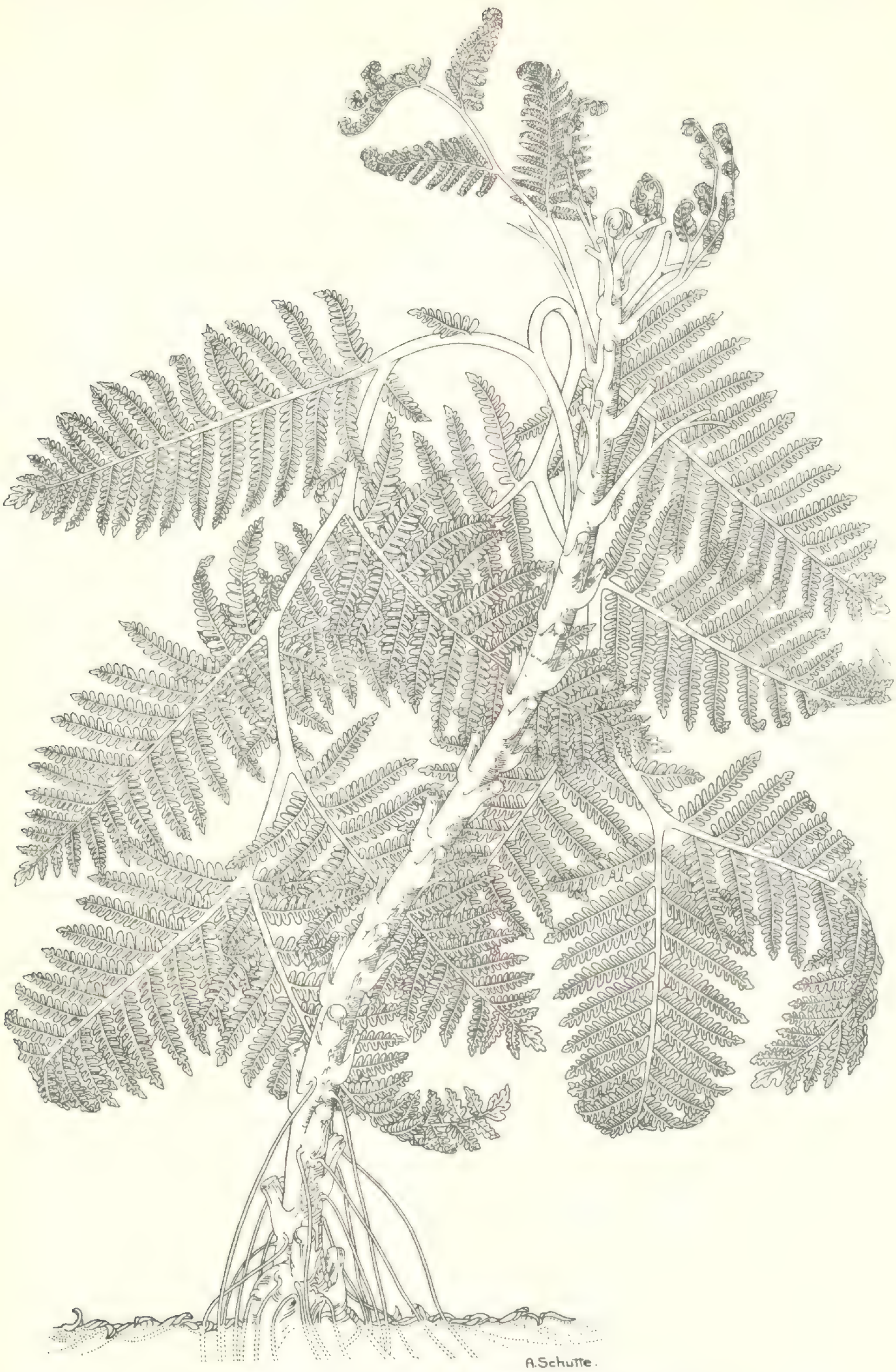


AMERICAN CARBONIFEROUS FLORAS. VII

Explanation of Plate

PLATE 13

Restoration of a *Medullosa* of the Anglorota group, based primarily on the stem and rachis remains of *M. Thompsonii*, together with associated foliage and root fragments. For further explanation see pp. 332, 334; x approximately 1/8.



ANDREWS—AMERICAN CARBONIFEROUS FLORAS. VII.

Explanation of Plate

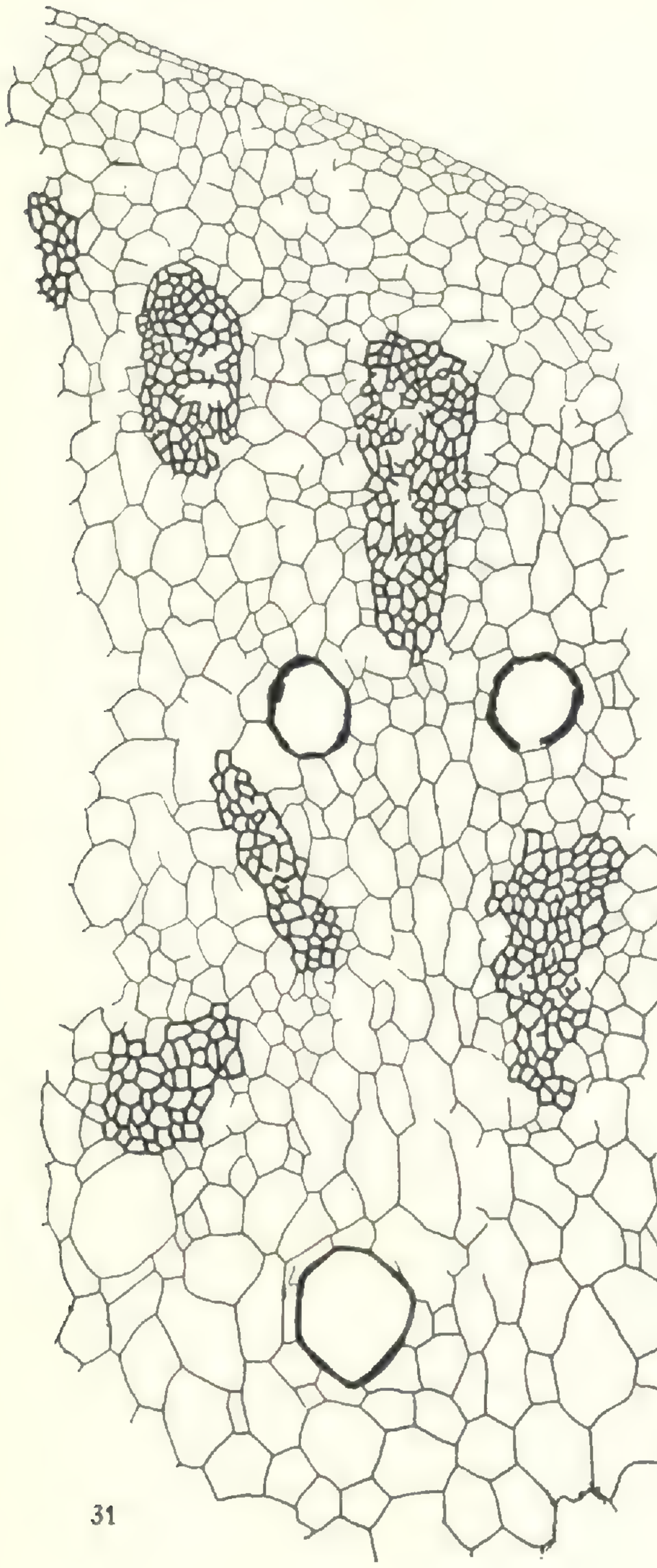
PLATE 14

Medullosa Thompsonii Andrews

Fig. 31. Outer cortex of the petiole shown in fig. 9. WCB426-T2, x 36.

Fig. 32. Outermost cortex and epidermis of the stem shown in fig. 1. A part of two fibrous strands are also shown. Slide No. 1364, x 65.

Fig. 33. Cortex of the stem as it appears between points *b* and *c* of fig. 1: ph, phellem; phg, phellogen; phd, phelloderm. Slide No. 1363, x 65.



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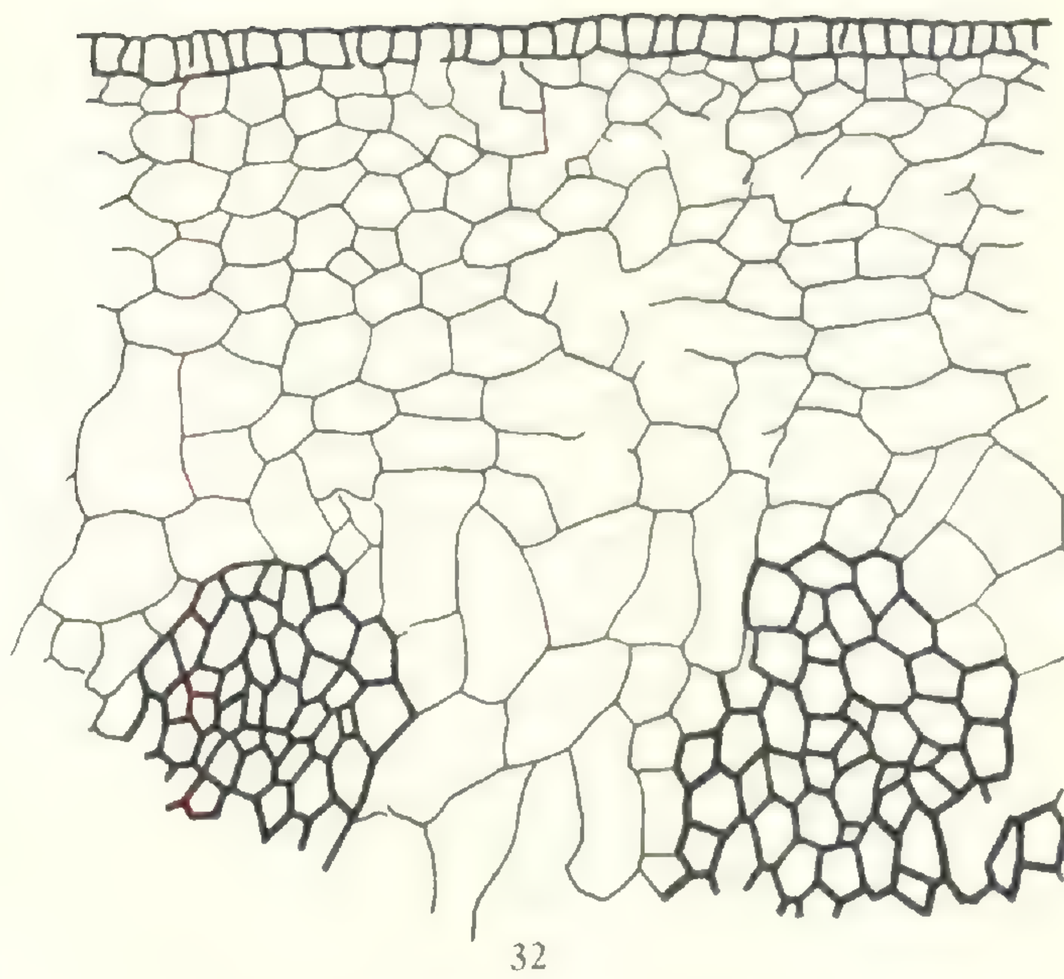

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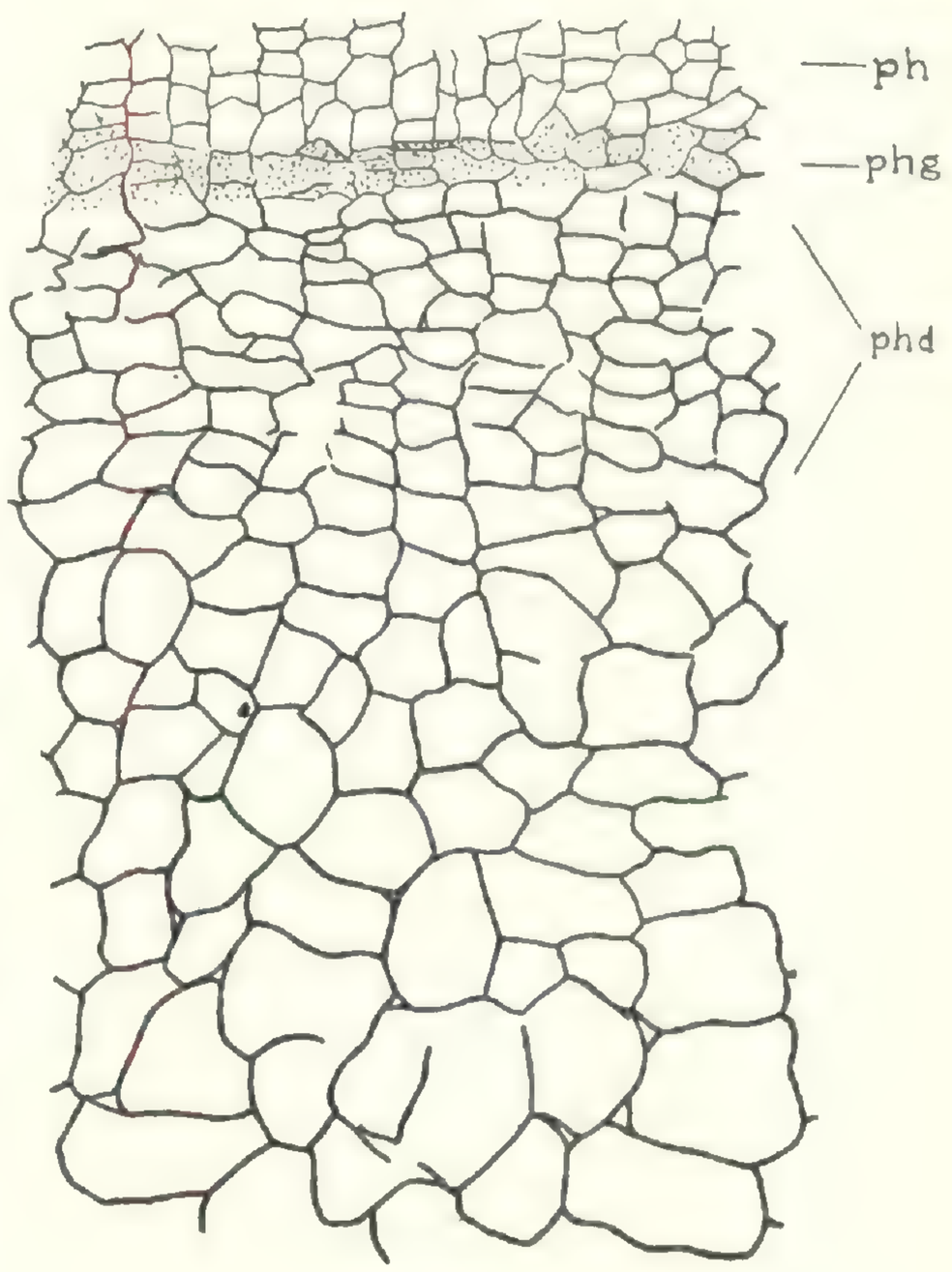

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ANDREWS—AMERICAN CARBONIFEROUS FLORAS. VII.

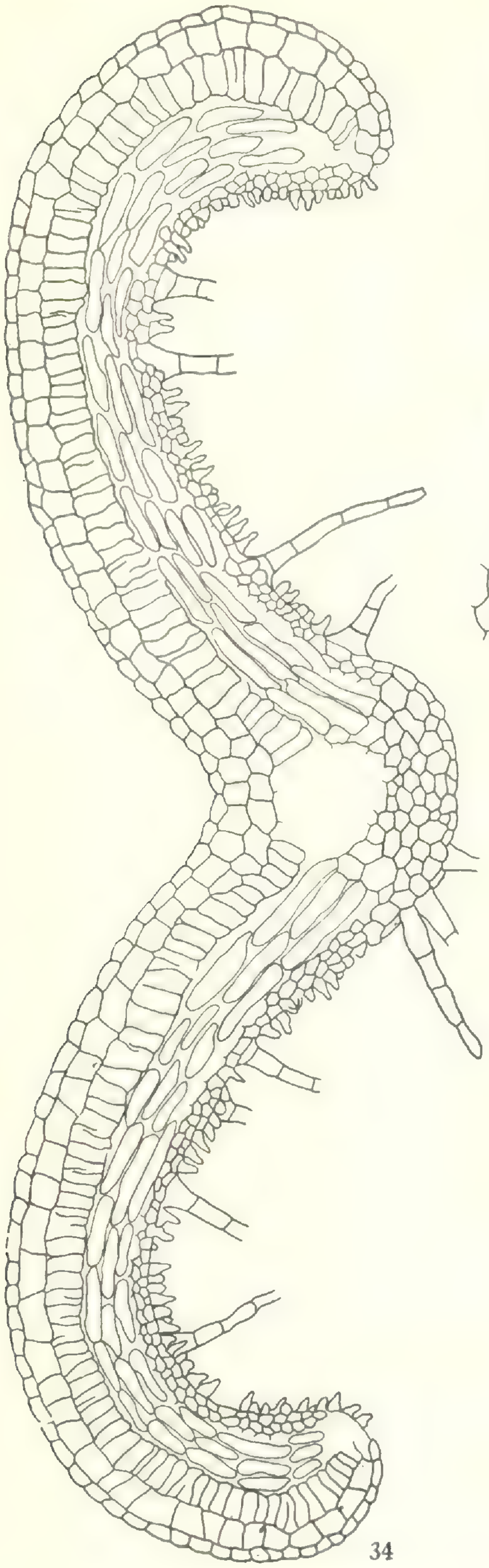
EXPLANATION OF PLATE

PLATE 15

Fig. 34. Reconstruction of *Alethopteris*-like leaflets found associated with the *Medullosa* stems and petioles, x 48. Explanation in text.

Fig. 35. *Myeloxylon Bendixenii.* Outer cortex showing the secretory canals exterior to each fibrous strand. WCB429-S2, x 42.

Fig. 36. *Schopfiastrum decussatum.* Diagram of the transverse section shown in fig. 18. Primary xylem stippled, secondary xylem indicated by radiating lines, and traces solid white; protoxylem groups of primary xylem and traces are shown by large black dots. Slide No. 1353, x 7.5.



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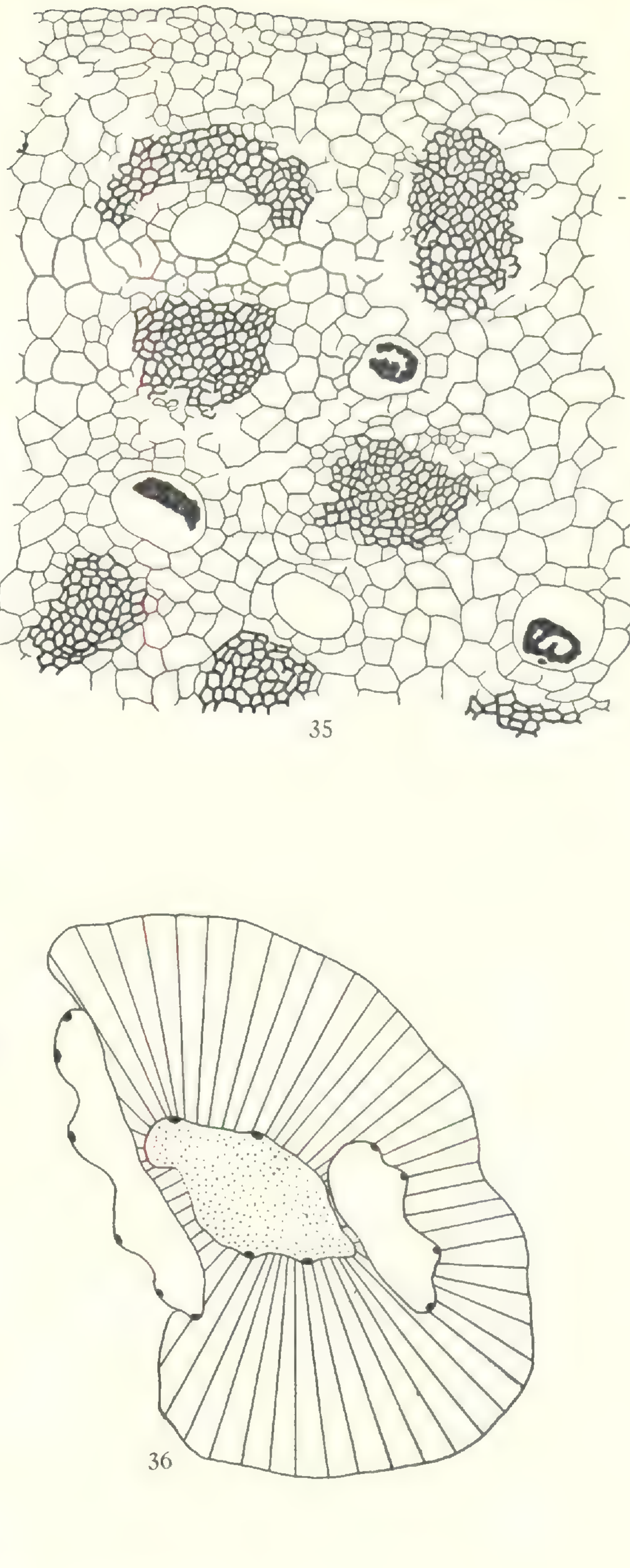

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ANDREWS—AMERICAN CARBONIFEROUS FLORAS. VII.

MAIZE IN THE YANHUITLAN CODEX

EDGAR ANDERSON

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The appearance of a definitive edition[1] of the Yanhuitlán Codex, under the editorship of Wigberto Jimenez Moreno and Salvador Mateos Higuera, gives us a unique opportunity to learn something about the maize of southern Mexico immediately after the Spanish Conquest. This interesting Codex, which fascinates scholars by its naive blending of native and European techniques, provides us with a good deal of incidental information about the yields and uses of maize at that time and place. In addition, some of the delineations of the maize plant are so realistic as to be useful scientific evidence.

Like many important codices, the Yanhuitlán Codex has had a checkered career, and its entire history is not known. The editors discuss the evidence in detail. The original of the document has been for a long time in the Academía de Bellas Artes in Puebla, Mexico. The exact date when it was placed there is not known, but in 1892 it was mentioned in a catalogue of the Mexican Exhibit of the American Historical Exposition in Madrid. Its history previous to this period and the reasons why it was brought to Puebla remain unknown. The Codex was written on paper of Spanish manufacture in the sixteenth century, and it bears a watermark used in Spain from about 1550 to 1570.

The town of Yanhuitlán, whose history the Codex records, is located in the Mixteca Alta half-way between Nochistlán and Tepozcolula in the present state of Oaxaca. As the name Alta indicates, this is a high, cold region.

The Codex is almost unquestionably of native workmanship. The drawings show both Spanish and Indian influence, and no Spanish-trained scholar of the time would have undertaken to write a history in such a form. The sketches are dated according to native pre-Conquest technique by characters and symbols identical with those used in pre-Conquest documents. Discussing the history of a Mixtec town, the work is annotated in Mixtec rather than in Spanish.

From an artistic point of view (as well as an historical), the drawings in the Codex are significant because they appear to have been sketched a very short time after the actual events occurred, perhaps in the very sight of the subjects depicted. On the other hand, the drawings show European influence as well. Pre-Conquest drawings have no perspective and solid figures are without shading to indicate contour. The perspective of buildings and moving figures in the drawings is imperfect, but it is frequently attempted and people are sketched in with realistic shading. The drawing was done with pen. Shading appears to have been added

[1] *Códice de Yanhuitlán.* 89 pp. 24 pls. Mus. Nac. Inst. Nac. de Antropol. e Hist. Mexico, 1940.

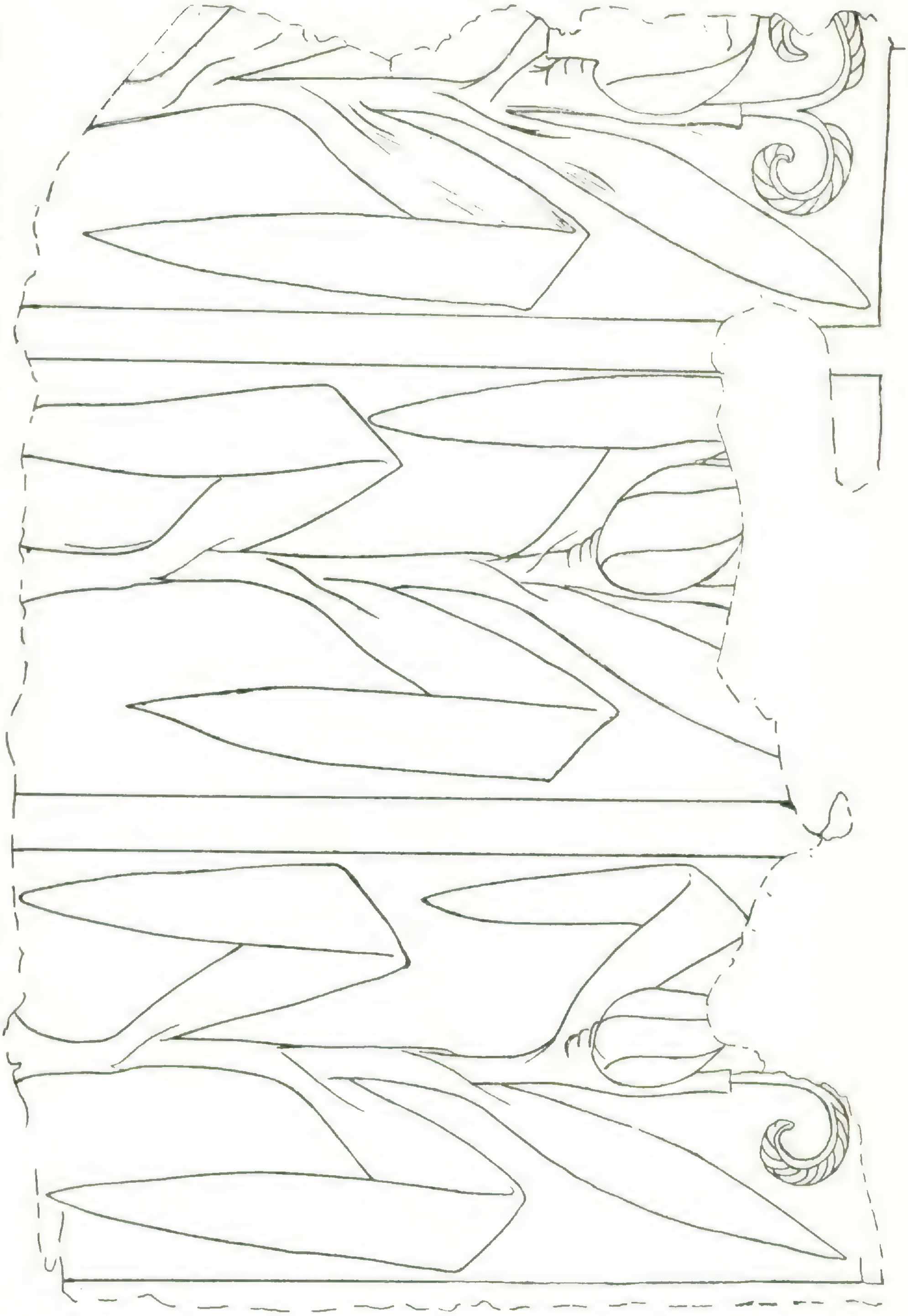

Fig. 1. A tracing of Lámina XVII. Broken lines represent torn edges of the original sheet. The drawing shows three plants of maize each with one ear at the left and the tassel above.

with *pincel* (artist's brush), and this process—used by the aborigines—leads one to believe that the author of the record was already a *tlacuilo* (Aztec scribe) at the time when Spanish culture arrived.

The realism of the drawings demonstrates fairly accurately what kind of maize was under observation by the artist. Mexico now has strikingly different types of maize in different parts of the country. Eventually from archaeological and historical evidence it should be possible to work out in considerable detail the history and origin of the different types as is already being done in other areas.[2]

The maize plants in this figure (pl. 16 and text-fig. 1) are distinguished by their broad, more or less bent leaves, by the short ears whose husks spring out sharply from the stem at almost a right angle, and by coarse tassels with few branches. All these features are characteristic of one of the commonest types of Mexican maize, the many-rowed, short-eared, dent-kernelled varieties which are centered on the region around Mexico City and which Anderson and Cutler[3] have provisionally named "Mexican Pyramidal." The semi-stylized drawings of the tassel have undoubtedly been influenced by the customary pre-Conquest glyph for the maize plant (a tracing is shown in fig. 2) and therefore may not be too diagnostic. The realistic treatment of the rest of the plant is, however, completely unlike anything in pre-Conquest documents, and is such an exact representation of present-day "Mexican Pyramidal" plants that it presents almost indisputable evidence that the artist had such a plant before him when the drawing was made.

Fig. 2. Pre-Conquest drawing of a maize plant (from the Codex Fejérváry Mayer). Note stylized representation of the three tassel branches. Compare with fig. 1.

The demonstration that in the late 16th century a Mexican Pyramidal type of maize was being grown in Oaxaca, where such types are to-day exceedingly common, would not be very significant if it were not for an important archaeological fact. The Zapotec funerary urns from near-by regions sometimes include representations of the maize ear in the head-dress of the main figure. These are frequently so stylized as to be useless in determining what type of maize was being grown at the time they were made. A whole group of them, however, are realistic, and many probably represent actual casts made from the ears themselves. Without exception these record a type of maize which is to-day either unknown or at least exceedingly rare in Oaxaca—one which looks different from Mexican Pyramidal varieties

[2] Carter, George F., and Anderson, Edgar. A preliminary survey of maize in the southwestern United States. Ann. Mo. Bot. Gard. **32**:297-322. 1945.

[3] Anderson, Edgar, and Hugh C. Cutler. Races of *Zea Mays:* I. Their recognition and classification. Ann. Mo. Bot. Gard. **29**:69-88. 1942.

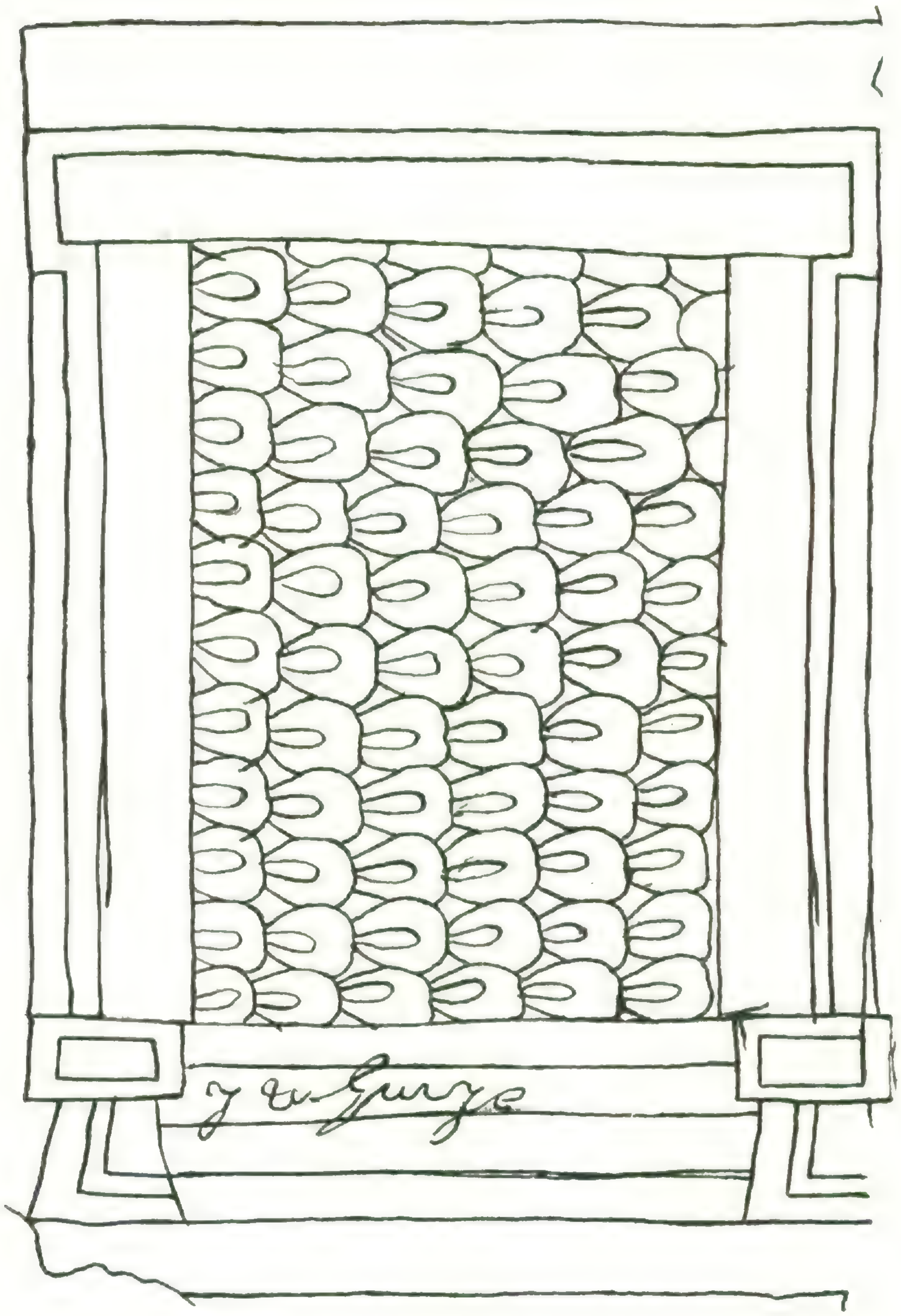

Fig. 3. Tracing of a portion of Lámina XI (see pl. 17) representing a granary filled with maize. The kernels are shown lying on their sides and are obviously derived from the pre-Conquest glyph for maize.

and suspiciously similar to the Basketmaker maize which was being grown in the southeastern United States at about the same period. By analogy with the types of present-day maize which resemble it, we would predict that the maize of the Zapotec funerary urns had narrow tough leaves which did not break readily, that it has long wiry tassel branches with at least 10 to 15 branches in each tassel, and that its husks were wrapped tightly around the ear at the base. Whether or not it was actually of this type, it could not by any stretch of the imagination have been borne on such a plant as the one so realistically depicted in the Yanhuitlán Codex. Such ears could never have been borne in these husks.

The following is a literal translation of the editors' description of the drawings of the maize plant:

> Three large rectangular drawings cover this page, but lower and side strokes cannot be seen because the two margins of the paper are lacking. Each of the sketches is a replica drawing of the one and same maize plant. If the plants were drawn with roots, as was the native custom, they cannot be seen because of the frayed margin. The bases of the stalks are broader than other parts, and the plants' corresponding leaves are perfectly drawn, some with their natural creases. An ear of corn covered by its husk is attached to the stalk near the top. The tips of the plant cannot be distinguished because the paper is torn there and the drawings are covered with adhesive tape to hold the page. But in the side sketches parts of the tassels terminating the plant can be seen. It is one of the peculiarities of these drawings that each stalk represents a cornfield during its stage of maturity. It was their custom to express in paintings one single thing to represent the whole.[4]

In addition to defining at least one of the types of maize under cultivation in Oaxaca in the late 16th century, the Yanhuitlán Codex gives us a good deal of incidental information about the culture and uses of maize at that period. The crudely drawn imperial granaries of the pre-Conquest Matricula[5] are replaced by more detailed and realistic delineations. The editors describe these drawings as follows:

> [The drawings on Lámina XI] represent four granaries drawn like four buildings on platforms, each of which has a high-stoop opposite with four or five treads. Between the lines there is written repeatedly a Mixtec inscription: "*ytu huiyo*" meaning "corn field." [According to the vocabulary of Alvarado, *huiyo* means "corn germinated without sowing it."] On the two sides which define the width of the treads, wooden beams are drawn at the bottom in trapezoidal form, perhaps in order to indicate descent—with parallel lines sketched in as decorations at the edges. These appear to indicate decoratively the formation of ascending and descending steps. And toward the top resting on this section are rectangular roofs somewhat narrower than the parts at the base. In the same direction as these beams, plain window posts arise without other decoration than parallel lines which seem to simulate more elaborately embossed borders. These extend around the lintels resting directly on top of the window posts. As the document lacks colors and these sections do not have description to indicate the material used in its manufacture, it is impossible to determine if the granary is made of wood. But this is probable since it is known that beams were most commonly used in these structures. The roof must have been flat since the top is not drawn otherwise. The interiors of these granaries are full of shelled corn drawn as kernels of large size perfectly recognizable. It should be noted on this page that these four granaries full of corn are the product of four sowings which the Indians of Yanhuitlán are obliged to cultivate in order to satisfy what was prescribed in the required valuation of the governor, Don Domingo, "by reason of its value during the time which he held it." The valuation was made by the Viceroy, Don Antonio de Mendoza, October 26, 1548, and, after estimating

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[4] *Op. cit.* Lámina XVII, pp. 64-65.

[5] Anderson, Edgar, and R. H. Barlow. The maize tribute of Moctezuma's empire. Ann. Mo. Bot. Gard. **30**:413-420. 1943.

other loans, it mentions: "They are to reap four more sowings of corn; two of these have 400 *brazas en quadro* each; the third, 300; and the fourth, 600."[6].

The widespread use of maize as a tax or tribute is referred to in the editors' study of the Codex:

> . . . In order for all the priests of S. Domingo to enjoy the fruition of this cornfield [in Yanhuitlán] . . . they will be given the harvests of wheat and corn for the sustenance of all the priests . . .[7]
>
> . . . [The Indians] will make four more plantings of corn, two of which will reap 400 *brazas en quadro*, a third, 300, and the fourth, 600.[8]
>
> . . . Each of them pays in taxes 782 pesos and half in gold dust, and they plant 15 *hanegas* of wheat; . . . 700 tortillas of maize and 30 *huesos* and a half *hanega* of maize . . . This is a fertile land for corn and wheat . . .[9]

It is interesting to note from the Codex that maize was carried in packs in exactly the manner which prevails today in parts of Mexico. The authors comment on Lámina XII as follows:

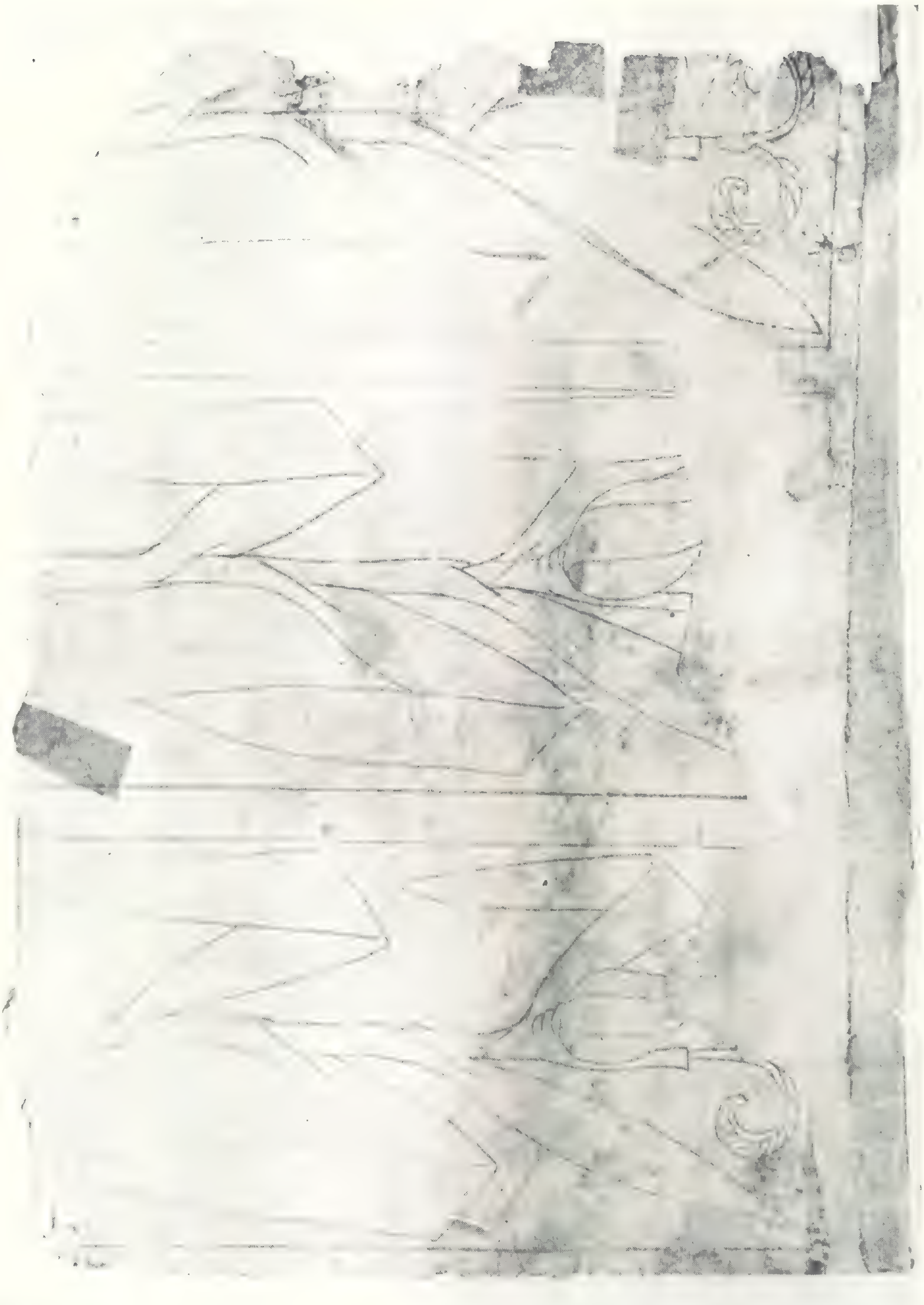
> . . . In the center of the upper half, one sees the picture of a *tameme*, a native carrier whose body is covered by nothing other than a *maxtlatl*, without any decoration. This, together with the duty he is performing, indicates his low condition. One of the ends of the cloth covering him falls to the front, and the other is knotted at the back of the belt then hangs down. The carrier's burden consists of a thinly woven sack full of shelled corn which he carries by using a *mecapal* [tump line, *i. e.* a long rope used by porters with a flat band fitting over the forehead]. This object, still used today, consists of a fiber woven from maguey in the form of a band, and variable in width as much as ten centimeters; on its ends it has some handles made of textile material. Cords are attached to these tying the weight.[10]

[6] *Op. cit.*, Lámina XI, pp. 61-62.
[7] *Op. cit.* p. 33.
[8] *Op. cit.* p. 36.
[9] *Op. cit.* p. 33.
[10] *Op. cit.* p. 61.

EXPLANATION OF PLATE

PLATE 16

Lámina XVII of the Yanhuitlán Codex, showing three plants of maize, each one of which probably symbolizes an entire field (see text). A tracing of this *lámina* is shown in text-fig. 1.

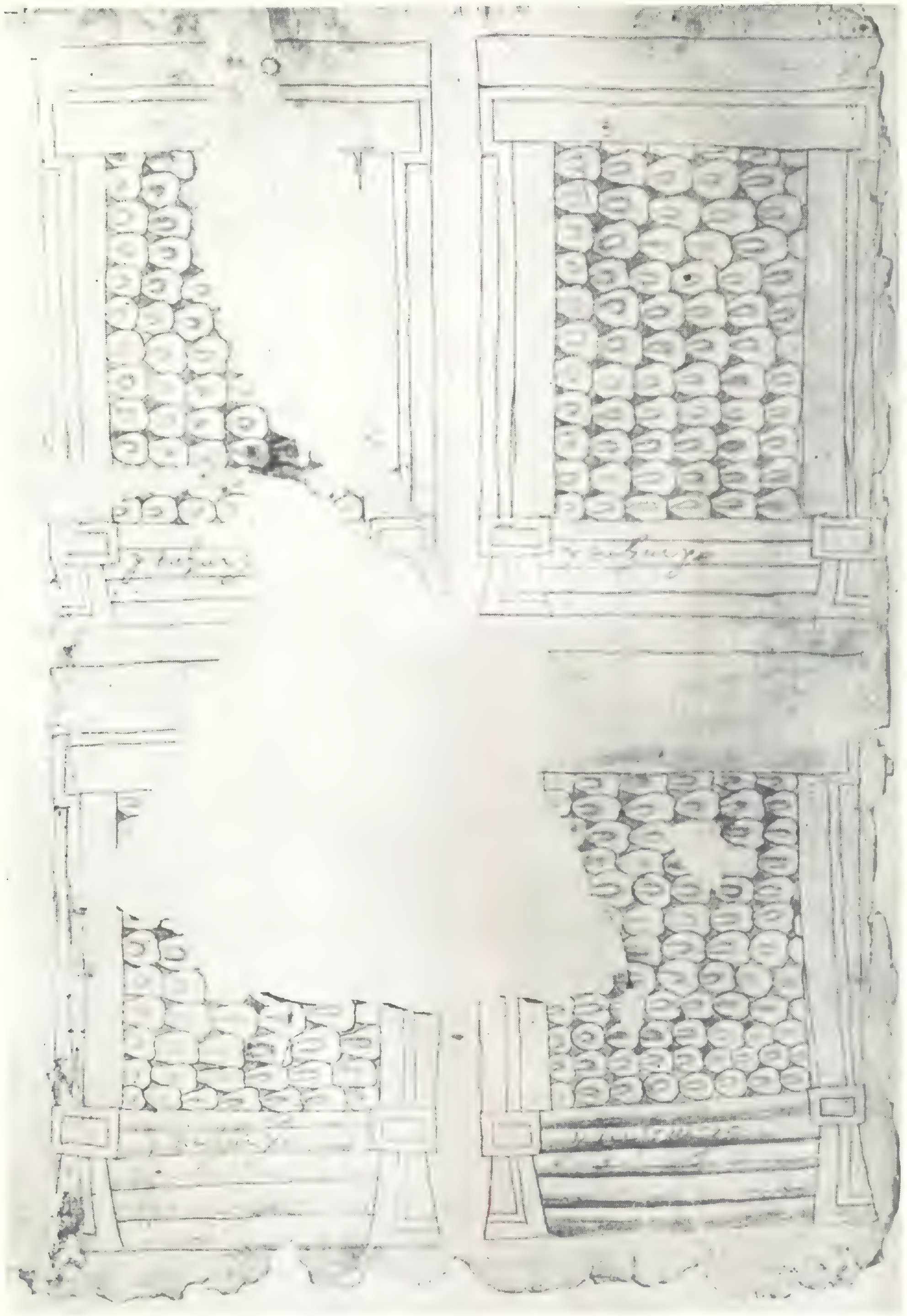


ANDERSON & DINAN — MAIZE IN THE YANHUITLAN CODEX

EXPLANATION OF PLATE

PLATE 17

Lámina XI of the Yanhuitlán Codex. Four granaries *(trojes)* filled with maize. A tracing of the upper right-corner is shown in text-fig. 3.



ANDERSON & FINAN — MAIZE IN THE YANHUITLAN CODEX

NOTES ON SOME NORTH AMERICAN ASCLEPIADS[1]

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Associate Professor in the Henry Shaw School of Botany of Washington University

ASCLEPIODORA DECUMBENS WHEN TREATED AS AN ASCLEPIAS

The first results of my studies of the American Asclepiads were embodied in a "perspective" of the genera (Ann. Missouri Bot. Gard. **28**:193-244. 1941), prominent within which was a drastic reduction of the segregate genera *Acerates, Asclepiodora, Solanoa, Podostigma,* and many others, to *Asclepias* L. That treatment was intended as a reformation of the generic lines, and although numerous illustrative changes of nomenclature were introduced, keys to all the included species, and their synonymy, were not provided. As I pointed out at the time, appropriate combinations under *Asclepias* already were available, with relatively few exceptions, some of which were offered thereupon. One case which I neglected, however, was that of the familiar "Antelope-horns" of the southwestern States, *Asclepiodora decumbens* (Nutt.) A. Gray. In the interval since the 1941 publication, I have received a number of requests for the correct name of this plant when considered under *Asclepias,* from botanists who are aware of the earlier homonym *A. decumbens* L.

At first glance, an early name for *Asclepiodora decumbens* seems to be provided in *Asclepias brevicornu* Scheele (Linnaea **21**:756. 1848) as indicated arbitrarily by 'Index Kewensis', but further consideration shows that this disposition probably is incorrect. The description of Scheele's plant, *Römer s. n.* from the neighborhood of New Braunfels, Texas, paradoxically points to synonymy with the fortunately earlier *A. longicornu* Benth., which is common in the same vicinity. Association of *A. brevicornu* with *A. longicornu* is suggested strongly by the following excerpts from the original diagnosis of the former: "*Corolla . . . laciniae . . . primo patulae, dein reflexae. Corona . . . cuculli speciosi aurantiaci falcati oblongo apice rotundati medio utrinque auriculati processum brevissimum includentes gynostegio longiores . . .*" Scheele further remarks concerning the chief distinguishing characters of his species: "Eine prachtige, durch das sehr kurze Horn und die schönen, sichelförmigen, beiderseits geörhten Klappen ausgezeichnete Art, mit Keiner andern zu verwechseln!" It is obvious, at least, that Scheele did not consider his *A. brevicornu,* indicated by 'Index Kewensis' as a synonym of *Asclepiodora decumbens,* as at all closely related to the next species that he proceeded to describe, *A. longipetala,* based upon a Lindheimer collection from New Braunfels fortunately available in the herbarium of the Missouri Botanical Garden. This happens to be the common plant known as *Asclepiodora viridis*

[1]Continued from Ann. Missouri Bot. Gard. **31**:369. 1944.
Issued September 15, 1945.

(Walt.) A. Gray *(Asclepias viridis* Walt.), which anyone will grant to be extremely closely related, indeed, to *Asclepiodora decumbens*.

An examination of the diagnosis of *Asclepias brevicornu* shows further the impossibility of classifying it as an *Asclepiodora*, particularly in the characters of the reflexed corolla and the "schönen, sichelförmigen, beiderseits geörhten Klappen [hoods]." These immediately place the plant as a member of Kunth's old genus *Otaria*, exemplified by such species as *A. longicornu*, *A. nyctaginifolia*, *A. Emoryi*, and *A. subulata*. Only the first of these could have been collected in the vicinity of New Braunfels, with the possible exception of *A. Emoryi*, the hoods of which are too short to coincide with Scheele's diagnosis. The discrepancy of Bentham's and Scheele's names apparently for the same species is explained by the allusion of the former to the strikingly elongate hoods and of the latter to the short, adnate horns.

Since *Asclepias brevicornu* Scheele is not available as a name for *Asclepiodora decumbens* when treated as an *Asclepias*, and since none other exists, it becomes necessary to coin a new name (in allusion to the popular name):

ASCLEPIAS **capricornu** Woodson, nom. nov.

Anantherix angustifolia Raf. Atl. Journ. 146. 1832, non *Asclepias angustifolia* Schweig.

Anantherix decumbens Nutt. in Trans. Amer. Phil. Soc. **5**:203. 1837, non *Asclepias decumbens* L.

Anantherix Nuttalliana G. Don, Gen. Hist. 4:146. 1838, nec *Asclepias Nuttalliana* Tor., nec A. Gray.

Acerates decumbens (Nutt.) Dcne. in DC. Prodr. **8**:522. 1844.

Asclepiodora decumbens (Nutt.) A. Gray, in Proc. Amer. Acad. **12**:67. 1876.

Asclepias decumbens (Nutt.) K. Sch. in Engl. & Prantl, Nat. Pflanzenfam. 4^2:239. 1895, non L.

Asclepias capricornu occupies a wide territory of the southwestern United States from central Kansas to east-central Texas and westward to Arizona and southern Nevada. Over this area it is not a phenotypic unit. Preliminary studies have satisfied me of the presence of well-defined continuous obliquely stepped clines (J. S. Huxley, in Bijdr. Dierk. $\mathbf{27}^{E}$:494. 1939) of an east-west direction in at least four essential characters of the plants. These are found in the leaf base, presence or absence of a naked peduncle, hood color, and follicle surface. Unfortunately, three of these are essentially qualitative in nature. However, when assigned arbitrary scores and plotted, a biologically significant discontinuity is discovered for all four characters centering in western Texas. In the zone of intergradation, the cline is conspicuously steep, and, as I have said, occurs at almost the same gradient for all four characters.

Such being the case, it appears appropriate to indicate two subspecies: an eastern (including the typical element of the species) characterized by obtuse leaf bases, sessile inflorescences (i.e., immediately subtended by leaves), light-colored hoods, and more or less spiny follicles, and a western, characterized by narrowly acute leaf bases, pedunculate inflorescences, dark-colored hoods, and

smooth follicles. I expect to investigate the relationship of these subspecies more fully when conditions permit.

ASCLEPIAS CAPRICORNU ssp. **capricornu** Woodson, ssp. nov.

Speciei elementum typicum; foliis basi vulgo obtusis nisi truncatis; inflorescentiis sessilibus vel subsessilibus; coronae cucullis vulgo pallidis; folliculis plus minusve spinosis.

ASCLEPIAS CAPRICORNU ssp. **occidentalis** Woodson, ssp. nov.

Plantae speciei habitu congruentes sed foliis angustioribus basi plerisque anguste acutis; inflorescentiis plus minusve valde pedunculatis; coronae cucullis plus minusve saturate purpureis; folliculis laevibus.—Exemplum typicum: NEVADA: CLARK: Pine Canyon. Roadside near stream. With *Juniperus utahensis* and *Pinus monophylla*. Alt. 1800 m. May 24, 1940. *I. W. Clokey 8613* (Herb. Missouri Bot. Gard., TYPUS).

(To be continued)

Annals
of the
Missouri Botanical Garden

Vol. 32 NOVEMBER, 1945 No. 4

THE YUCCA PLANT, *YUCCA FILAMENTOSA*, AND THE YUCCA MOTH, *TEGETICULA (PRONUBA) YUCCASELLA* RILEY: AN ECOLOGICO-BEHAVIOR STUDY[1]

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Of all the delightful treatises in the field of natural history, none, I dare say, surpasses the one by Charles V. Riley on the life-history of the Pronuba moth and its relation to the pollination of the flowers of the Yucca plant. Observations and experiments on this relationship by Riley and the botanists, George Engelmann and William Trelease, covered a period of twenty years. During that long time, they repeatedly uncovered the intricate and almost unbelievable details of the behavior of the moths at the flowers, and often the work was conducted in the presence of friends and colleagues. Their findings may be verified to-day, by any one with a flashlight, during the blooming period of the plants.

Riley published short papers from time to time as the investigation progressed, and finally put the whole story together in the 'Third Annual Report of the Missouri Botanical Garden' in 1892, under the caption, "The Yucca Moth and Yucca Pollination." The paper is charmingly written, replete with beautiful drawings made by Riley himself, and is to-day regarded as one of the classics of natural history. This treatise points out to us that nowhere else do we find such hand-and-glove interdependence of flower and insect. Neither plant nor insect could perpetuate itself without the other, for the Yuccas depend solely upon the moth for pollination, and the larval moths in turn depend solely upon the ripening seeds for food.

In order to insure the development of the seeds so that the larvae may have food, the mother moth actually packs pollen into the stigmatic opening of the pistil. By this act she also accomplishes fertilization in a very difficult flower

[1] My specimens of the Yucca moth were kindly identified by Mr. August Busk of the U. S. Department of Agriculture. Other insects mentioned were identified by others whose names appear in brackets throughout this paper.

which cannot be pollinated by wind or by bees. Since the larvae eat only a small portion of the growing ovules, the plant enjoys the benefit of an ample crop of seeds.

The most striking part of Riley's paper, from the standpoint of insect psychology, is the behavior of the mothers at the flowers. However, from the standpoint of ecology and evolution, the most exciting part is the discovery that the short-lived, non-feeding moths come upon the scene simultaneously with the opening of the flowers, which also are short-lived—a perfect adaptation in time of each to the other. The origin of this adaptation has never been adequately explained. Like all problems which touch upon the fascinating subject of origins, it is difficult to investigate. I have, however, made a feeble attempt at such a study, as the later pages will show. I have also verified much of Riley's work, and in the course of observations have stumbled upon additional facts on the behavior and the ecology of both insect and plant.

BEHAVIOR OF THE MOTH

First, let us review briefly Riley's discoveries on the interdependence of moth and flower.

The adult moths appear just as soon as the flowers open. Being silvery-white, they enjoy a marked amount of color protection when within the white flowers. The flowers have the most attraction during the first and second nights of their opening, probably because their fragrance is strongest at that time. The moths, small and delicate as they appear, are hardy and are strong fliers.

The mother, when ready to oviposit, gathers a ball of the sticky pollen from the anthers. In order to do this, she uncoils her tongue over the anther and stretches out to the fullest extent her maxillary tentacles. Then, by a series of forward and backward movements of the body, she scrapes the pollen with her palpi toward the tentacles. She goes from one anther to another, sometimes to as many as four, until she has a large load. This she kneads and shapes into a ball, and holding it firmly under her chin, she runs about until she finds a flower which is suitable for ovipositing. Having found one, she seeks a favorable point on the pistil and, thrusting her lance-like ovipositor into the soft tissue of the ovary, conducts the egg to its destination. No sooner is the ovipositor withdrawn than the moth thrusts a portion of her pollen-ball into the stigmatic opening and works her head rapidly "with vigor that would indicate pleasure and purpose" in packing it down. She makes every effort to force the pollen into the tube, often using her tongue to thrust it forward into the cavity.

The one load of pollen serves for several fertilizations. Each time she deposits an egg in the pistil, she repeats this process of cramming pollen into the stigma, and this is true of any subsequent eggs which are deposited in the same pistil—one fertilization for each egg deposited is the rule.

There is no other method by which larvae hatching from these eggs are assured of food, and Nature has provided no other means of pollination for this plant but this fantastic one. The behavior of the insect is all the more impressive when one remembers that she herself gets nothing at all from the flower. In fact, she has no means of imbibing food, and yet she goes through these intricate movements solely to supply, or rather to manufacture as it were, food for her young.

Riley was highly fascinated by the wonders which unfolded before his eyes, and he says:

> We have in the structures and functions which are so characteristic of this Yucca moth, admirable adaptations of means to an end . . . . . There is between Pronuba and its food-plant a mutual interdependence which at once excites our wonder, and is fraught with interesting suggestions to those who are in the habit of reasoning from effect to cause . . . . . The peculiar structure of the flower which prevents self-fertilization, though on a superficial view it strikes one as a disadvantage, is, in reality, a benefit, as the value of cross-fertilization has been fully established; while the maxillary tentacles of the female moth are very plainly an advantage to her species in the 'struggle for life'; and it is quite easy to conceive, on Darwinian grounds, how both these characteristics have been produced in the course of time from archetypal forms which possessed neither, and in reality we get a good insight into the process in studying the characteristics of other species of the family Prodoxidae. These peculiarities are, moreover, mutually and reciprocally beneficial, so that the plant and the animal are each influenced and modified by the other, and the same laws which produced the beneficial specialization of parts will maintain them by the elimination of all tendencies to depart from them.

The Yucca Plant and Its Flowers

The Pronuba moths are on hand during the very first evening that the flowers are open, and may be seen within the blossoms during the entire blooming period. Even at the end of the season, the last and only flower remaining on the stalk may be full of them. Flowers and insects appear as if by magic at the precise moment. If they did not do so, the perpetuation of both species would be defeated. This meeting at the right time is not an occasional coincidence, but evidently is the result of long years of "give and take", "come and go", and "trial and error"—natural selection at work eliminating year after year the offspring of one or the other that came upon the scene too soon or too late. Eventually the time period of both, in hand-and-glove fashion, became fixed in heredity, and the natural selection that brought on this condition of coincidental appearance maintained them by the same methods.

Before seeking to discover what influences are responsible for this coincidental appearance, one should first know something about the flowering habits of the plant. The flowers of *Yucca filamentosa* bloom in Missouri in June of each year, and remain open for only a short time. I have compiled in the table data on various aspects of the flowers' biology for nine consecutive years, 1934 to 1942 inclusive.

Year	1st flowers to bloom on author's terrace	1st flowers to bloom elsewhere in the neighborhood	Date flowers began to decline	End of blooming period	Number of flower stalks on plants on author's terrace	Number of days plants in flower
1934	June 5	———	———	———	———	———
1935	June 17	June 15	June 30	July 12	38	27
1936	June 6	June 4	———	———	———	———
1937	June 13	June 9	June 29	July 5	70	22
1938	June 4	May 31	June 22	June 30	53	26
1939	June 11	June 7	June 28	July 2	40	21
1940	June 13	June 11	June 27	July 2	58	20
1941	June 2	May 31	June 14	June 26	105	24
1942	June 2	May 30	June 19	June 29	28	27

The table shows that the flowers bloom from 20 to 27 days each year. The first flowers opened between June 2 and June 17, and the last flowers disappeared between June 26 and July 12, inclusive. The variations of the opening and closing dates, while apparently slight, are of vast importance, since the short-lived moths must, without fail, match this blooming period by their own appearance. This leaves us wondering what causal factors have sharpened Nature's mutual adjustment to so fine a point. Is this simultaneous development due to some environmental factors, such as early or late spring, wet or dry periods, sunshine or shade? And do these conditions influence in some way the flower-bearing stalks and the insects in the earth as well? If they do, it will go well with both, but if one or the other puts in its appearance too soon or too late, both flower and insect must die without leaving progeny.

The Yucca plants which grow on my terrace and are somewhat shaded by trees during part of each day, I regard as growing in semi-shade. There are also a large number of plants growing in a sunny open field near by. For a number of years, I have noticed that flowers appear on the plants in the sunny location earlier than they do on my semi-shaded terrace. In the third column of the table, you may see that the flowers bloom from one to four days earlier in the open sun than they do in the semi-shade.

On the other hand, in a heavily shaded estate not far from my home, I find the Yucca flowers appearing still later than those on my semi-shaded terrace. For

example, in 1941 the plants on my terrace began to flower on June 2, and on June 8 had 105 stalks, 82 of which were then in flower. On June 8 I examined the stalks in the heavily shaded estate, and counted 25, none of which bore a flower. Two days later, June 10, all of the 105 stalks on the terrace bore flowers, and of the 25 plants in the heavily shaded garden, only five stalks had open flowers, and then only from one to three to a stalk. Another instance in point: On July 2, on the highway near DeSoto, Missouri, I noticed a large number of Yucca plants in a heavily shaded cemetery, all of which were in full flower; a mile down the road, however, in a sunny farmyard, a similar lot was all through flowering and all stalks bore large green seed-pods.

Thus we see the effects of three environmental situations—shade, semi-shade and sunshine—on the blooming propensities of Yucca. Sunshine and shade are undoubtedly factors, within limits of course, in regulating the appearance of Yucca flowers. Riley seems perturbed when he finds the Yuccas blooming two weeks later in Philadelphia than they do in near-by Washington. He says there appears to be some irregularity in the blooming time of these plants. Evidently, what he did was to observe one set growing in the sunshine and another in the shade. In the light of the behavior of our neighborhood Yuccas, sunlight and shade would account for the irregularity which Riley observed. He does not tell us if there was also a similar irregularity in the appearance of the moths.

The Stalk, the Flower and the Seed-pod

Before we go into the details of the regularity and irregularity of the appearance of the moths, we must have some knowledge of the day-to-day progress of the growth of the plant, especially the development of the flower-stalks, the opening of the flowers, and the ripening of the seed-pods. These details are interesting from the standpoint of flower ecology, and have some bearing on the life of the Pronuba moth.

Here, as an example, are the happenings on my terrace in 1941:

May 18. The flower-stalks, light-green, tender and asparagus-like, are pushing their heads up from the center of the plants.

May 27. They are now half-grown, and number 105.

June 2. 11:30 A. M. The flower-stalks are full-grown but bear no flowers. A careful search is made about the plants for any Pronuba moths that may be lying in wait for the flowers to open; none are found.

Same day, 10 P. M. Some time during the afternoon, 15 flowers have opened, and these are on four stalks. An examination at 10 P. M. revealed 52 Pronubas crowded into them. The temperature is 72° F. No moths were lurking about the plants or buds when I examined them at noon, and now there are within the flowers 21 males and 31 females. Their distribution is as follows:

				Males	Females
2	flowers,	each	with	1	1
1	"	"	"	4	4
2	"	"	"	0	1
1	"	"	"	0	5
1	"	"	"	2	1
5	"	"	"	1	2
1	"	"	"	1	3
1	"	"	"	3	1
1	"	"	"	4	3
Total 15	"	"	"	21	31

June 3, 10 P. M. Three additional stalks now have flowers, and all of these harbor moths, some of which are in copula.

June 5, 10 P. M. A total of 30 stalks now have flowers, and almost all of them contain moths. Observing them until midnight, I find them nervously walking and flying about the flowers, evidently in search of newly opened ones that may not yet have served as a repository for eggs.

June 7. A total of 53 stalks now have flowers.

June 8. A total of 82 stalks now have flowers.

June 9. A total of 98 stalks now have flowers.

June 10. All 105 stalks now have flowers.

June 14. Some of the first stalks to bloom now have dead or discolored flowers, and also small, green seed-pods are beginning to form on several of them.

June 21. The first stalks to bloom are now in complete decline, but on the others, flowers continue to open.

June 23. The flowers on about 85 per cent of the stalks are rapidly declining, and some of them bear green seed-pods two inches long, thus giving evidence that Mother Pronuba has done her work well.

June 26. Only three stalks now remain that have flowers, and these total only eight. Three of these contain moths, probably newly emerged, for their wings are clean and fresh.

June 29. Only one flower now remains open, and it has attracted to itself a Pronuba, probably, too, the last one of the summer. So here ends the season of Pronuba moths and Yucca blossoms. They have appeared on the scene simultaneously, have played their brief drama strenuously, and now are no more, but the bountiful crop of green pods filled with ripening seeds and growing larvae assures continued life to both species.

July 7. The pods are large, and the seeds within them are ripening; the larvae are feeding on the seeds and are becoming large and fat.

July 16. Some of the pods show spots on the outside. These spots of discolored tissue thinly cover the exit holes in the pod-wall which were cut by the larvae in anticipation of their escape. This bit of foresight (if it may be so called) on the part of the larvae will be discussed later.

July 27. The green pods are rapidly turning brown and becoming hard, and the spots have now given way to full-sized openings from which the larvae are escaping and dropping to the earth.

August 13. The pods are becoming very tough, and the exit holes numerous, indicating that practically all of the larvae have emerged.

August 18. During the past few days, about 75 per cent of the pods have reached full maturity and have burst open, scattering the many seeds which were not eaten by the larvae. With the ripe seeds now disseminated over the surrounding earth, and a new generation of Pronuba babes snugly encased in silken cocoons in the ground, the activities of the whole year, all crowded into a few days, end, and for ten months life seems to have gone out. But with the advent of another summer the flowers and insects will spring into action *simultaneously* to repeat the drama.

The Adult Moth

The moths, as already stated, are to be seen within the flowers during the very first evening of their opening; they continue within the perianth during the entire blooming period of 20 to 27 days; and when one peeps into the last remaining flower at the end of the season he will be greeted by the disturbed moths flying into the air.

In the foregoing chapter the details of moth and flower relations for the year 1941 were given. Additional confirmatory notes are herewith presented.

In 1935, on June 16, there were 30 flower stalks on my terrace in bud, but a careful search about the plants revealed not one adult Pronuba moth. The next evening, three of the stalks bore flowers, within which 18 moths were counted. On the final day of their blooming, July 12, only one open flower remained, and crowded in it were 12 moths.

In 1937, on June 17, four days after the plants commenced to bloom, 243 flowers were counted, and the moths were estimated to number about 75; ten pairs of these were in copula.

In 1938, the first flowers opened on June 4. There were 14 of them on three panicles, and at 10 P. M. 55 moths rested within them. Some flowers harbored from five to ten, and others, of course, had none. The few remaining flowers at the end of the season, June 27 to June 30, all contained moths to the very end.

In 1939, the first flowers opened on eight panicles on June 11, and during the first evening each of them contained from one to four moths; during the last days of blooming, June 28 to July 2, there were moths in the few remaining flowers.

In 1941, on the morning of June 8, six days after the flowers had begun to open, I counted only five moths in 50 flowers. In the early afternoon, more flowers burst open, and the air was full of their fragrance. The small number of moths was perplexing, to say the least, but when I visited the flowers early that evening masses of silvery winged Pronubas were flying to the new flowers.

This gave me the first idea that the moths do not come up from the earth

immediately surrounding the plant and walk directly up the stalk to select a flower by the sense of sight. They are without doubt attracted to the flowers by the sense of smell, and the reason I found only 5 moths in 50 flowers was that either the flowers were so old that they had lost their fragrance, or the wind was in the wrong direction or not strong enough to carry the news to the moths lying in wait somewhere. This subject will be enlarged upon later. It is strange that the fragrance of the flower should attract these insects, since for themselves food is not their goal. The object of the quest for the female evidently is a place where she may, in ichneumon-fly fashion, deposit her eggs, and the goal for the male in the flowers can only be the presence of the female. It is a complicated situation indeed if the male responds, not to the odor of the female, but to the odor of the flower she frequents.

The moths of both sexes, easily distinguishable, are short-lived and take no food, the tongue having lost its function. (In the laboratory the males lived two to three days, and the females three to five days.) They spend their adult lives within the full-blown perianth. Mating takes place there, and the pairs remain together facing in opposite directions for from two to four hours. When disturbed, they often walk about slowly without separating.

The behavior of oviposition is startling enough, but that of gathering pollen (is any other moth known to gather pollen?) and deliberately using it to impregnate the ovaries of the plant, thereby creating—instead of merely gathering—food for her young, seems as incredible, as one author puts it, "as a tale of Munchausen."

The aforementioned behaviors are mainly biological and psychological, but equally startling are the ecological facts connected with the simultaneous appearance of the moths and flowers. Has the plant adapted the time of the opening of its flowers to the appearance of the moth, or is it the other way around? Is the coincidence that we witness the "end stage" of a long series of steps of "give and take", "come and go", in the evolution of this phenomenon?

We know little of these steps, but we may some day discover them by the historical or by the comparative study of the near relatives of Pronuba. To digress for a moment, various not-far-distant relatives of Pronuba are given to many singular and astonishing ways of making a living for themselves and their young, and perhaps Pronuba has come by her eccentric habits honestly. To mention but a few of the queer things that her relatives do, one may cite the clothes-moths, the wax-moths, the fur-sloth moths, the cattle-horn feeders, the owl-pellet feeders, the pigeon-trash feeders. Some lepidoptera are even parasitic on bumblebees and wasps, and one species has even gone so far as to be an internal parasite of certain Coccidae. Being unwilling to wait until a study could be made of Pronuba's relatives, past and present, I proceeded to carry on experiments to see what external environmental conditions may be responsible for the delicate adjustment of insect to flower in point of time.

The Experiments

The larva, having completed its feeding within the Yucca pod, drops to the ground, penetrates the earth a few inches and spends the winter underground in a cocoon of silk that it has spun for itself. Late in the spring, a short time before the opening of the Yuccas, the larvae transform into pupae. The pupa is heavily armed with spade-like dorsal spines with which, at the proper time, it forces itself out of the earth. When on the surface, it speedily transforms into a winged moth ready almost immediately to pollinate the flowers.

Suspecting that the larvae may be influenced by temperature conditions, simple experiments were set up to learn what one could about it. The larvae, when full fed or nearly so, were removed from the pods and placed in tin cans with loose soil; they readily buried themselves in it. The cans were tightly covered but were aerated occasionally, and the soil was moistened four or five times during the winter.

Experiment I.—Several hundred larvae were placed in tin cans on July 28, 1937, and kept in a room during the winter where the temperature varied from 42° to 60° F. A careful record was kept of the dates the adults emerged the following spring, and are as follows:

1938		Number of moths
May	17–18	55
	19–20	21
	21–22	18
	23–24	36
	25–26	49
	27–28	5
	29–30	36
June	1	41
	3	3
	6	29
	7	8
	14	13
Total		314

The data are extremely interesting in connection with the dates of flowering of the Yuccas for that year. This period for 1938 was from June 4 to June 30, 26 days. Now we see in this table that three-fourths or more of the moths emerged too early to do the plants or themselves any good. If the moths in their natural habitat emerged in the same way, it would be woe to both insect and plant. Evidently the emergence was influenced by temperature conditions, and, as one would expect, the occasionally heated room (at least it was warmer than the outside earth in which the moths normally spend the winter) stimulated development, and the moths emerged too soon.

In 1941-1942 similar tests were made under different conditions.

Experiment II a.—This is a repetition of Experiment I, and was made solely for the purpose of serving as controls of Experiments II b and II c, to follow. The dates when the adults emerged and their numbers follow:

1942		Number of moths
May	19–20	6
	21–22	3
	23–24	10
	25–26	22
	27–28	14
	29–30	12
June	1–2	17
	3–4	8
	5–6	8
	7–8	5
	9–10	4
	18	1
July	1	1
	4	1
Total		112

Here the conditions were the same as in Experiment I, and the dates when the 112 adults emerged coincide very nicely with those in that experiment, except for two stragglers in July.

Experiment II b.—On the same day that controls were set up in Experiment II a, another one was set up as II b, with this important difference: the cans were kept outdoors in an open barn, where the temperature was practically the same as that outside. The conditions of this test are closer to those of the natural hibernating quarters of the moths in the earth, but still are not quite the same.

The dates of the emergence and the numbers were as follows:

1942		Number of moths
June	1–2	10
	3–4	16
	5–6	52
	7–8	43
	9–10	10
	11–12	4
	13–14	4
	15–16	2
	17–18	3
	19–20	2
	21–22	0
	23–24	3
	25–26	3
	27–28	2
Total		154

Thus 154 moths emerged from cans in the barn from June 1 to 28; 139 during the first half of June, and only 15 during the last half. By comparing these dates with controls kept indoors (Experiments I and I a) we see that temperature is a potent factor influencing emergence. The larvae that were kept in cans in the cold barn emerged in line with those hibernating in the earth, quite in time to pollinate the Yucca flowers.

The flowers that year were open from June 2 to 29, and the first moths to emerge from my experimental cans came on June 2. They kept emerging thus from day to day during the entire blooming period of the Yuccas, with not a

moth overstaying the flowering period by even a single day. So well did this experimental emergence coincide with the normal emergence (and also with the opening of the flowers) that when my moths were liberated near the plants they joined their comrades on the flowers, as though they were "native here, and to the manner born."

Experiment II c.—This test did not turn out well, evidently due to bad technique, but is included here more as a matter of record than for scientific yield. At the time Experiments II a and II b were started, 411 larvae, in six cans, were buried a few inches below ground near the Yucca plants. The cans were covered tightly, but either because of too much moisture or the lack of air, the mortality was enormous.[2]

The cans were exhumed on May 20 and examined every day for emerging adults, but only five came to the top, and all from one can—one on June 9, 3 on June 13, and one on June 25. These dates were all in line with the flowering period of the plants, and indicate at least that the conditions in the ground differed very little from those in the barn.

As shown in these experiments, the influence of temperature on emergence gives us an answer, in part at least, to the question we must ask ourselves when noting the difference in blooming time for plants growing in sunshine and in shade. How can emerging moths meet this erratic blooming behavior? Reasoning by analogy from the experiments, the answer is that when low temperature or lack of sunshine retards the blossoms, it affects likewise the emergence of the moths. A portion of ground heavily shaded by trees would take a longer time to warm up than a portion in the sunshine. This would retard the moths in the earth to an analogous extent that the shady cool environment above ground retards the flowers. By the retardation of both to a similar extent, nothing is lost in the end, and flower and insect meet and function normally.

How the Moths Come to the Flowers

As has already been stated, no moths are to be found near the plants when they are in bud, even a day or two before opening, but moths are often abundant in the flowers during the first evening of their blooming. I have always suspected that the moths bury themselves in hibernation near the growing plants, emerge from the ground some time before the flowers open, and lie in ambush, as it were, ready to fly to them at a moment's notice. In these studies I have learned that the caterpillars wander about for some time before entering the earth, and later as adults they are attracted to the flowers (as the following details will show) by the fragrance carried on the wind.

In 1935 the first flowers, three in number, opened on June 17. It rained all day on June 18, and the temperature remained around 58–60° F., and no other buds opened. June 19, with the temperature about 60–62°, 84 flowers opened,

[2] The cans were filled three-fourths full of soil, but those in the room and in the barn, which could more readily be handled, were often aerated.

but up to 7 P. M., not a moth was to be seen within a perianth. When I returned at 8:30 P. M., however, I found the air filled with the silvery-winged moths flying to the newly opened blossoms, while many were already settled within them, and others were walking from flower to flower, evidently seeking something "just a little bit better." I returned again at 10 P. M., and found the flight over, and from one to four moths in more than a third of the flowers, many of them in copula. It is plain to see in this case that the temperature of the day before did not deter the moths, for it differed little during the two days, but rather the lack of odor upon the wind caused their delay. It is very interesting to note also that even though the fragrance was on the air all the afternoon, the moths waited until after dark to respond to it.

During the next few days I could not visit the flowers until 10 P. M. I found newly opened blossoms from day to day, with many moths crowded into them. But on June 25, I was on hand earlier and again saw the moths flying to the flowers as they had done a few days previously. They wasted no time hovering before the flowers, but flew directly to them with a display of much nervousness and settled into them at once. They are strong fliers for so small an insect, and with my flashlight I could see them coming from the south. It was unfortunate that I could not discover from what distance they flew.

Another bit of evidence that Pronubas fly to the flowers from a distance was noted in 1938. A lawn in the town was leveled and resodded. Without touching the Yucca plants on the place, the workmen removed several inches of surface soil, thereby destroying any Pronuba larvae that might be hibernating there. The plants bore an abundance of flowers the following summer, but later not a stalk among them had seed-pods.[3] In 1940, they also produced flowers and also many seed-pods. The moths that effected the pollination must have flown there from the population on my premises, the nearest supply, and that about 1,100 feet away. They evidently had followed the trail of odor borne by the wind. Why did they do so in 1940, and not in 1939? My answer is, reasoning from analogy,[4] that it is quite likely the wind was not favorable in direction or in strength for carrying the flower odor to the places where the moths were.

Riley says (l. c., p. 122): "I have often been struck with the power which the moth has of detecting isolated plants blooming for the first time remote from other plants . . . . a fact which indicates that, where abundant, in addition to her ordinary more sedentary duties, she takes long reconnoitering flights."

In summary, I may say that Pronubas fly against the wind on the trail of the fragrance of the Yucca flowers, where they proceed promptly to the business of egg-laying. Pronuba moths do not fly at all hours of the night, but only between 8:30 and 9:30 P. M. This rhythmic periodicity is also found in certain species of fireflies and certain Saturniid moths, each species having its own set period for flight some time between twilight and dawn. The Pronubas often run

[3] The flower stalks shrivel when the flowers are not pollinated.

[4] The sex attraction and rhythmic periodicity of Saturniid moths. Acad. Sci. St. Louis, Trans. **26**:81-221. 1929.

restlessly from flower to flower; when this occurs, it is because there are too few newly opened flowers at hand, and the old ones have lost their attractiveness. I have frequently seen a moth inspect several flowers before selecting one in which to oviposit. Riley says that the stigmatic opening closes when once eggs are deposited in the pistil; perhaps this is a sufficient signal for the moth to seek a favorable place to oviposit elsewhere.

Unlike other moths, Pronubas are not attracted to light. The lighted windows of my home, only 40 feet from the terrace, have never attracted them. Only when they escape in the laboratory, where the dazzling light is very near, do they circle around the electric bulb in a confused manner.

The Larvae

Like the adult Pronuba, the larvae are quite hardy and can stand a lot of rough handling. Riley likewise found them so, for he says:[5] "It is the hardiest larva I have had to do with, and will not only repeatedly mend its cocoon when it is cut or torn, but when extracted from it, will survive for months if kept in a tight vessel." More than that, I find that the panicles of seed-pods may be cut from the plant and transported in the automobile for long distances. During the shake-up, many larvae fall from the pods and may later be picked up from the floor of the car. When the more mature ones of these are placed in cans of loose dirt, they will develop into normal adults.

The tiny, white, newly hatched larvae feed upon the white ovules. As the seeds become larger and darker, the larvae too grow larger, fatter and more colorful, and finally when the caterpillars reach full growth the color is red, tinged with green.[6] They eat the tender centers of a row of tightly packed seeds, destroying from 18 to 25 in the process. The tough rims of the seeds are not eaten, but serve (closely packed together as they are) as a wall of the cell-like domicile while the larvae continue to eat their way through the compact row. In addition to being closely packed, the seeds are held together by strands of silk spun by the larvae, as well as by bits of excrement pushed to the far end of the tunnel. This makes a comfortable "cocoon" and is so tightly held together that the whole set of otherwise loose seeds may easily be removed as one mass.

In the darkness of its cell, the larva grows while it eats its way through its food-mass and enlarges its tunnel. But toward the end of its career, it exhibits a bit of behavior that seems to bespeak purpose as much as the adult action in pollination. When nearly mature, the caterpillar interrupts its feeding long enough to cut a hole in the outer wall of the pod to permit its later escape. It does this while the pod is still tender, and its jaws can crush the green tissue of the wall. If the caterpillar waited too long to bite this hole, the walls would be found to be too tough, and the insect would be entombed. This job neatly done, it resumes

[5] Sixth Rept. State Entomol. Mo. pp. 131–135. 1874.

[6] One sometimes finds full-grown caterpillars that are of a beautiful amethyst-green hue. In one lot of 80, three such were found. When they were brought to maturity, the adults differed in no perceptible way from the reddish-green ones.

its feeding.

This precaution, which so resembles foresight, is not the only commendable item of its behavior. When cutting this hole through the wall of the pod, it stops short when it reaches the thin green outer skin. Thus the hole is concealed from the outside, yet is easily broken when the larva is ready to emerge. This concealment of the hole might evade enemies, for birds occasionally break open the pods. But Riley says that the open holes permit moisture to enter, causing a growth of fungus which might be detrimental to the larvae.[7]

While normally the caterpillar eats the tender centers of its row of seeds, when it cuts the exit-hole for future use, it must, in order to reach the pod-wall, bite its way out through the outer edges of the row of seeds; also it must turn squarely at right angles to its habitual course. After these sharp digressions from its usual quiet life, it goes back to feeding. The little discs of skin, like tightly stretched drum-heads, covering the holes, often turn brown while the pod is yet green, and are tell-tale landmarks that point to a fat larva just beneath. The holes are small, and the fat larvae have to struggle to push through them when escaping. They do not just fall out of the holes, but wriggle through in what appears to be a painful ordeal, at last breaking the thin, outer skin of the pod as they come out.

After the larvae have dropped to the earth, they walk about for some time before burrowing into it. Those in the laboratory, when placed on loose soil, wandered about for several hours before crawling down into it. It was formerly thought that the larvae, falling from the pods, enter the ground near their own plant; but seeing the larvae busily crawl about in the cans of earth, and also later seeing the adults fly to the flowers from a distance, I concluded that the larvae travel some distance from the plant for hibernation.

Riley says that the larvae penetrate the ground five or six inches, but in my tin cans they went down into the loose soil from one to three inches. They spend the winter underground, and in the late spring they transform into heavily spined pupae. At a later propitious moment they work their way out of the ground, shed the horny covering, inflate the silvery wings, and are ready for the business of reproduction when the first fragrance of Yucca flowers permeates the air.

If the larvae are needed for experimental purposes, the pods must not be gathered too early, or the larvae will be underfed; on the other hand, if one waits too long, the larvae will have escaped into the ground. They should be gathered, as near as possible, just when they have finished feeding; and the tell-tale brown spots on the outside of the full-grown green pods indicate that feeding is nearly

[7] It is interesting to note that the larvae of the bogus Yucca moth, which has a common ancestral origin with Pronuba, behave in a similar fashion. They feed on the pith of the flower stalk, but before spinning a cocoon eat a passage-way to the outer covering of the stem. However, they leave intact the thin membrane on the outside, through which later as adults they escape. Those which emerged from dried stems gathered hereabouts proved to be, according to Mr. Carl Heinrich, *Prodoxus quinquepunctellus* Chamb., which he states is a synonym of *P. decipiens*.

over. If the caterpillars are taken before they are mature, the mortality will be great. In a lot of 300 larvae gathered too soon, there was a mortality of 95 per cent in my cans, while under similar conditions, in a lot carefully selected for complete feeding, 48 out of 50 larvae transformed into normal adults.[8]

Dates of the exodus of the larvae may vary slightly in different localities and in different years in the same locality. However, at Kirkwood, pods on about July 15–25 contain full-grown larvae, while perhaps ten days later, the holes will be open and the larvae gone. If the panicles are left on the stalk, the pods naturally dehisce. If they are brought into the laboratory, they harden prematurely, and this before the larvae within can provide the escape-holes. The larvae are then prisoners, but they spin cocoons around themselves in their tunnels of half-eaten seeds and spend the winter in that way. A lot was discovered one spring, after having spent the winter within the pods in my cold barn, and they were found to be in good condition. Later all became normal adults.

It is amazing that so few Pronuba moths are parasitized. With several thousand developing in the laboratory, not one parasite issued from them. It is not due to the fact that the larvae are distasteful, for I have fed dozens of them to Polistes wasps, which in turn fed portions of the meat to their larvae. They were accepted as food by larval ant-lions also.

The larvae are generally free from enemies, excepting for a bird occasionally breaking into a pod, or a mouse eating the larvae along with the seeds in the laboratory. However, Riley has found ants destroying the larvae in the ground.

The larvae live and grow in these apparently air-tight pods, and the number per pod varies. There is no relation between the size of the pod and the number of insects feeding within it. In 1937, near the end of the season I gathered 10 panicles bearing 316 pods. Dissecting the pods, I made a count of the larvae within them, with the following results:

Number of larvae in each pod	Frequency	Total number of insects
0	3	0
1	12	12
2	19	38
3	48	144
4	60	240
5	64	320
6	54	324
7	16	112
8	24	192
9	8	72
10	7	70
11	0	0
12	1	12
Total	316	1536

[8] The tin cans were about three-fourths filled with loose earth and covered with tin lids, but they were aerated and lightly moistened during the season.

The 316 pods harbored 1,536 larvae, or an average of nearly 5 per pod. But it is interesting to note that more than two-thirds of the pods harbored from 3 to 6 larvae. In an extreme case, one pod had 12 larvae, and in this pod every seed was destroyed. In each of the 15 pods containing 9 and 10 larvae, only a few seeds (from 6 to 15) remained uneaten. In most of the other pods, there remained hundreds of good seeds ready for dissemination.

An interesting item in the table is the fact that three pods containing seeds had no larvae in them. This was to be expected, since these three pods bore no constrictions. Riley has shown that the deposition of the eggs in the pistil is responsible for the constrictions in the middle of the maturing pod. If the constriction is slight, only one or a very few larvae is likely to be found within the pod; if it is deep, many may be expected. Riley was able completely to eliminate the constrictions by pollinating the flowers by hand.

Riley found also, and my observations substantiate this, that no other insect is able to pollinate the Yuccas, since pollination requires that the sticky pollen be tightly packed in the stigmatic opening.

Of the 316 pods here examined, only 3 bore no larvae, and these had no constrictions. In the light of Riley's observations, my only explanation is that either the mother's ovaries were depleted, in spite of which she packed the stigmatic opening with pollen, or her instinct went so far astray that she packed her little bundle of dynamite into the stigma but omitted to place the egg in the pistil. Similar miscarriages of instinct often occur among the solitary wasps, where plentiful food is provided for the young and the egg is not deposited.

INSECTS OTHER THAN PRONUBA TAKEN ON THE PLANTS

Riley presents a list of several other insects which are to be found about Yucca flowers and plants.[9] He found positively that these insects had no hand in the pollination of the flowers; that office is performed by *Pronuba yuccasella* alone. I have found other insects about the plants, and I also have ascertained that these have no part in the transfer of pollen. A list of insects and their behavior follows, and I should like to mention that only three of my records are the same as Riley's; these three are marked with an asterisk.

BEETLES[10]

**Carpophilus melanopterus* Ev. [E. A. Chapin]. These beetles were present each year, and sometimes six or eight were to be found in one flower. Sometimes they shared a flower with several Pronubas. They were present during the entire blooming period of each year, but especially they were noticeable in the very first flowers on the night of their opening, and often did damage by eating portions of the pistil or by biting their way into the very heart of the unopened bud.

Obrium maculatum Oliv. [W. S. Fisher]. Only one beetle of this species was taken; it was on the outside of the flower on June 25, 1937.

[9] Footnote in Fifth Rept. Insects Mo. p. 154. 1873, and in Amer. Assoc. Adv. Sci. 29:626. 1880.
[10] Names in brackets are those of persons who identified the insects.

Anthobatula trifasciata Melch. [H. S. Barber]. Only one specimen of this rove-beetle was taken; it was inside a flower, June 25, 1937.

**Chauliognathus pennsylvanicus.* The Pennsylvania soldier beetle was found each year about the leaves and within the flowers.

Coccinella novemnotata Hbd. [E. A. Chapin]. During the blooming season of 1935, many of these beetles, some of them in copula, were on the flowers. They were present on the stalks before the flowers opened, and remained through the blooming season.

Coleomegilla fuscilabris Muls. [E. A. Chapin]. One beetle seen on an unopened flower bud.

Trichiotinus piger F. [E. A. Chapin]. Only one of these Scarabidae was taken; it had its head deeply buried in a Yucca flower.

PLANT-LICE

Plant-lice, *Aphis rumicis* L. [P. W. Mason], are always abundant each year on the green flower stalks, although some years their numbers are much reduced by the aphis-lions. They usually collect on the bracts before the buds open and remain on them long after the white petals have fallen, often damaging the unopened buds. They feed on the juices of the plant, and appear to be just as abundant at the beginning of the season as at the end. They are often attended by the ant, *Formica fusca* var. *subserica* Say [M. R. Smith].

ANTS

When the tender flower stalks thrust up their asparagus-like heads, and later when the flowers are in bloom, one may often find aggregations of *Formica fusca* var. *subserica* Say upon them. They no doubt have been attracted to the plants by the plant-lice, but they have also been seen licking the exudations from the unopened flower buds.

Two other species of ants, *Monomorium minimum* Buckley [M. R. Smith] and *Penolepsis (Nylanderia) pavula* Magr. [M. R. Smith], were often seen on the flower-stalks, but what their interests on the plant were has not been ascertained.

APHIS-LIONS

The aphis-lions, *Chrysopa nigricornis* Bur. [A. B. Gurney], appeared in great numbers during certain years. At such times the plant-lice were greatly reduced. The females have often been observed depositing their stalked eggs on the plants at night.

HONEYBEES

*Honeybees, *Apis mellifica*, are always to be found about the flower-stalks, but they are seldom inside the blossoms. They usually content themselves with gathering the excretions on the outside of the base of the flowers. When they lap up the invisible excretions with protruding tongue, their abdomens pulsate rhythmically.

FLIES

Syrphus torvus O. S. [C. T. Greene]. These flies were often seen in company with the honeybees, lapping the exudations at the base of the flowers and also from the outside of the petals. Sometimes they fall prey to the flower spider, *Misumenops asparatus* Hentz. [E. B. Bryant], which often hides among the petals.

Allograpta obligua Say [C. T. Greene]. This fly was taken from the jaws of the above-mentioned spider in the center of a flower.

LEPIDOPTERA

Peridroma margaritosa Haw. [Carl Heinrich]. A caterpillar of this Noctuid species was seen eating into a flower-bud.

BUGS

Lygus pratensis oblineatus Say [H. G. Barber]. Occasionally a bug of this species was seen feeding on an unopened flower-bud.

Lopidea instabilis Reut. [H. G. Barber]. Seen occasionally feeding on flower-buds of the plant.

Leptocoris trivittatus Say [H. G. Barber]. A few nymphs taken from the plants during the blooming period in 1939.

Halticotoma valida Reut. [H. G. Barber]. This insect, known as the Yucca bug, has appeared on the plants on my terrace in such numbers during certain years as to injure them and reduce the number of flower-stalks. During 1939, only 40 flower stalks appeared (against 105 in 1941), and the flowers on each stalk were very few. Not one blossom was free of the bugs. Their sucking also produces numerous spots on the leaves, and not a leaf was free of these spots.

This enormous population in 1939 was evidently due to my having neglected to remove the fallen leaves that had accumulated about the plants for two years. The plants, however, recovered quickly when the accumulated debris was destroyed and the bug population thus reduced. After this had been done in 1940, the flower-stalks in 1941 numbered 105, all of which flowered heavily. The infestation appeared only on my neglected plants; other plants in the neighborhood were not noticeably infested. The bugs evidently find favorable winter quarters among the dead leaves, and spend their entire summer lives on the Yucca leaves, for in 1939 they were as abundant in October as they were in May.

INTERPRETATIONS OF PRONUBA'S BEHAVIOR

From the standpoint of comparative psychology, the behavior of Pronuba is of outstanding importance, and many students of behavior have sought in one way or another to explain the thorny problem of her actions. For example, McDougall[11] captions his discussion of the subject "Purely Instinctive Behavior," yet he is far from clear in throwing any light on the matter of the origin of these instincts. After describing the behavior of the moth at the flower, he says:

> Nature has so constituted the moth that she performs this cycle of nicely adjusted actions, essential to the continuance of the species, shortly after emerging from the chrysalis, when

[11] Outlines of psychology. pp. 74, 76. 1923.

she cannot have acquired any knowledge of the flower or of her grub and its needs. This is a fine example of the working of a chain instinct. Each step in the train of action brings the moth into a new situation in which new stimuli affect its sense organs. Why not be content to suppose, with the mechanists, that each step is simply a reflex action to some new stimulus . . . . . Consider a single step in this behavior, the placing of the egg in the one position in all the world where it can develop, this is among the ovules of the flower. Even if we assume that odor emanations from the ovules exert some tropic influence on the moth, it is obvious that this will not suffice to determine the placing of the egg in the right spot. That can be effected only under the guidance of a multitude of simultaneous and successive sense stimuli; and these must be not merely summated but rather synthesized and related to an appreciation of the shape of the parts of the flower concerned. In other words, the response of the moth to the flower is a perceptual response, not a mere reaction to a stimulus.

When one tries to find the meaning of "perceptual response" in his book, one is referred in the index to "Perceptual response to instinct" on page 99, but there we read the meaningless jargon which runs as follows:

> Instinctive activity is normally initiated by an activity of perception, more or less complex; the capacity for this activity is given in the innate constitution of the animal, and is an essential part of the total instinctive disposition (or instinct) as the capacity to execute the train of bodily movements which catch our eye.

It seems to me, however, that if the action of the moth is a *perceptual response*, then it is not an instinctive one, but rather more or less akin to discriminating behavior. Fearful of crediting Pronuba with psychic attainments of too high an order, McDougall, in my opinion, gets nowhere in his attempt to explain the insect's behavior.

Wells, Huxley and Wells likewise take a shot at Pronuba's behavior, and are likewise parsimonious in interpreting her actions in ovipositing. They say:[12]

> The impossibility of there being knowledge behind instinct is perhaps most prettily illustrated in the well-known case of the yucca plant and its moth, *Pronuba* . . . . . The association is one of mutual benefit, a reproductive symbiosis; the action of the female moth in putting the ball of pollen on the pistil seems admirably purposeful, just as her care not to kill the goose that lays the golden eggs, by only introducing three or four grubs into each flower-capsule, seems admirably calculated. But when we reflect that the mother moth dies before the seeds mature, and that the moths of the next generation have never seen a yucca in flower before they began their business of pollen-gathering and egg-laying, it becomes obvious that foresight and reason can play no part in the instinct—quite apart from the fact that experiments have decisively shown that no insect is capable of drawing such conclusions as the moth would have to draw if it were really being intelligent on the facts presented to it. We have no more right to suppose that the moth is being purposeful and intelligent in its actions than the yucca is being purposeful and intelligent in growing a pistil with a cup at its tip to receive the pollen; or, to confine ourselves to the moth, we have no more reason to find proof of intelligence in its actions in putting the yucca pollen in the proper place than in its growing the special appendage with which to manipulate the pollen.

Their parsimony goes still further when they say an instinct "is the outcome of the animal's nervous construction, as the leg and its working is the outcome of its mechanical construction. It is a bit of nerve-clockwork."

The statements by Wells, Huxley and Wells have the advantage of logic, but also the limitations of laboratory study. The authors go as far as they can in the generality that an instinct is the outcome of the animal's nervous constitution (which may or may not be true, because for all we know, the animal's nervous

[12] The science of life, p. 1153. 1929.

constitution may be the outgrowth of its psychic life, just as the mechanical construction of its leg may also be the outcome of its movements), but that is beside the point, since they have said nothing to bring us any nearer to an understanding of how all this came about.

Riley, on the other hand, takes a more magnanimous view of Pronuba's psychic qualities. He goes quite far in his anthronomorphic explanation, which is probably the result of his having spent twenty years observing the behavior of these silver-winged moths in the field:

> The pollen grains would not adhere by chance to the rolled-up tentacles, and we have seen how full of purpose and deliberation Pronuba's actions are. It may be that all her actions are the result merely of "blind instinct", by which term proud man has been wont to designate the doings of inferior animals; but no one can watch her operations without feeling that there is in all of them much of purpose . . . . . Nor can I see any good reason for denying these lowly creatures a degree of consciousness of what they are about, or even of what will result from their labors. They have an object in view, and whether we attribute their performances to instinct or to reason depends altogether on the meaning we give to those words. Define instinct as "congenital habit" or "inherited association" or, as I prefer to characterize it, as *the inevitable outcome of organization* [italics Riley's] and most of the doings of the lower animals may justly be called instinctive; but the instinctive and reasoning faculties are both present, in most animals, in varying proportion, the last being called into play more especially by unusual and exceptional circumstances, and the power which guides the female Pronuba in her actions differs only in degree from that which directs a bird in the building of its nest, or which governs many of the actions of rational man.

Coquillet, to quote from Lovell[13], is even more positive than Riley, for he regards the behavior of Pronuba as a purely intelligent act, saying: "There appears to be no doubt that she is in possession of the fact that unless she did thus pollinate the flower, there would be no seed pods for her offspring to live on."

Riley, as you have seen, grants to Pronuba a higher degree of psychic ability, and he does so evidently because he repeatedly observed how full of purpose and deliberation her actions are; but when he, as well as Coquillet, credit the moths with a consciousness of what will result from their labors, they merely indulge in a guess, for who can know what goes on in the heads of these creatures!

However, one must admit that there are in the insect world numerous analogous cases where the participants likewise act as if they knew, and knew very well, what would be the end result of their labors. Whenever I see Pronuba deliberately pounding the pollen into the stigmatic opening, other brilliant behaviors come to my mind. Who can deny, for instance, that the Empis fly does not realize to what purpose he dances before the female with the marriage offering of a captured may-fly which she is to suck during the process of mating; or who will doubt that the queen bumblebee has some consciousness as to what purpose she broods her eggs when, hen-like, she keeps them warm day and night until they hatch; or the male butterfly of the genus Belenois[14] to what purpose he strokes the wings of the desired mate; or the saw-fly, *Perga lewisii*[15], the end for

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[13] The flower and the bee. p. 144. 1918.
[14] Carpenter, G. D. H., A naturalist on Lake Victoria. p. 223. 1920.
[15] Carpenter, G. D. H., The biology of insects. Chap VIII. 1928.

which she strives when she watches over the eggs and later follows the young about as they feed, often covering them with her body to shield them from enemies and protect them from the sun; or the earwig, *Anisolabis mortima*,[15] when she cleans her eggs by rolling them in her mouth, and watches and guards them, as well as the young, when they are born; or does the bug, *Aepophilus bonnarei*,[15] when she gives the warning taps with her antennae which sends her young scampering for cover; or certain agricultural ants when they carry in their jaws on their marriage flight a pellet of fungus to start new gardens? And I cannot but recall my own observations on the intricate behavior of cockroaches,[16] in depositing and concealing their egg-cases. Many other examples could be cited.

But even if it is true that Pronuba's behavior is purely instinctive, we must admit that it could not possibly have always been so, for even an instinct must have had a beginning at some time. There is a first time for everything, and in the vast sweep of evolution, somewhere, sometime, certain especially endowed individuals, perhaps spurred to frantic exertion by some life-and-death stress, made unusual use of their faculties and adopted new ways with the flowers. The fact that a species performs a highly complicated and effective course of action, even though that course of action may now have become crystallized into instinct, points clearly to a line of progenitors who were versatile and were not afraid to try something new. It is an especially significant fact that relatives of this moth display an astonishing variety of outlandish accomplishments (mentioned elsewhere) which would justify our contention that the little Pronuba came from an "Edwards family" and not a "Jukes" in the insect world.

One may say in conclusion that if we wish to accord to present-day Pronubas a grain of intelligence, it is with the understanding that a great part of their actions are based on a well-developed set of instincts which were probably acquired bit by bit through the ages. On the other hand, branding their behavior as instinctive does not by any means preclude an ability occasionally to mix with it a bit of original variation, or a grain of something akin to intelligence. It may even require a modicum of intelligence to know when and where to make the best use of an equipment of instincts.

The Evolution of the Interrelationship

Both Yuccas and Pronubas, says Dr. William Trelease[17], are undoubtedly of recent geological origin; and the progenitors of the Yucca originally had spreading stigmas, and were also slightly entomophilous flowers pollinated by hymenoptera, diptera, or lepidoptera, which were attracted by the secretion of the septal nectar glands.

> With the consolidation of the stigmas, however, insects visiting the flowers for this nectar became inefficient pollinators, as may be seen when such insects enter the flowers of the existing Yuccas for the little nectar that is still produced; hence, with an economic reduc-

[16] See article, "How the cockroach deposits its egg-case; a study in insect behavior." Ann. Ent. Soc. Amer. **36**:221–226. 1943.
[17] Ann. Rept. Mo. Bot. Gard. **4**:217. 1893.

> tion of the secretion of these glands, may have come an addition to their function to that normally borne by the stigma, in an increase in its secretion, so that the visitors, laden with pollen unconsciously accumulated while on the flower, should further visit the stigma on which some of their burden might be rubbed while they were feeding. During this stage of its evolution the plant appears to have proved especially attractive to some small moth, perhaps fond of nectar, and with phytophagous larvae, which is to be regarded as the progenitor of the Pronubas. . . . .[18]

Riley, too, agrees that Pronuba and Yucca have arisen from simpler forms, for he says.

> The peculiar structure of the flower . . . . prevents self-fertilization; . . . . while the maxillary tentacles of the female moth are very plainly an advantage to her species in the "struggle for life"; and it is quite easy to conceive, on Darwinian grounds, how both these characteristics have been produced in the course of time from archetypal forms which possessed neither. . . .[19]

Since the structure of the insect has undoubtedly changed in the course of evolution, it is quite obvious that psychic changes have likewise occurred, and perhaps, after all, the brain and the mind of the free-flying Pronuba have played a more important role in the evolution of this singular relationship than has the brainless, immovable plant, which at most could have played only a passive role.

One can hardly assume that this mutual adaptation was a general merry-go-round process through the ages, each contributing equally to the other. The flower in the shadowland of its evolution could do no more than sway in the wind and abide its time, even as it does to-day. It had no choice in the selection of insects to perform the marriage rite, and could do no more than shed its fragrance on the passing breeze, and thus advertise its charms. The quality and condition of its charms, no doubt, varied over countless millenniums, and the insect was often compelled to choose or consciously select from among several variants.

The flower's important charms, in so far as Pronuba's behavior is concerned, are the stigma, the pollen, and the pistil, and from diversifications in these it had to select, for example: the stigmatic opening best suited to its pollen-pounding tongue; the pollen, dry, wet or moist, best suited to the carrying capacity or to the manipulating ability of its jaws; and the pistil best suited to the penetration of its peculiar ovipositor. In short, the blossom is selected by the insect and not the other way around. Pronuba has, in hammer-and-anvil fashion, hammered, let us say, the Yucca flower into what it is to-day, and the insect itself, in so doing, has undergone numerous changes—psychological and otherwise.

The Yucca apparently, as already suggested, has played but a minor part in the creation of the novel relationship, but a very important part, nevertheless—for if there were no Yucca-like flowers ever, there would not be (nor could there possibly be) the unique creature which we know to-day as *Tegeticula (Pronuba) yuccasella.*

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[18] *Ibid.* p. 219.
[19] Ann. Rept. Mo. Bot. Gard. 3:126. 1892.

A REVISION OF THE CENTRAL AMERICAN SPECIES OF SMILACINA*

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I. Introduction

The genus *Smilacina* was established by Desfontaines[1] in 1807, and was based on *Convallaria racemosa* L. Linnaeus[2], in the 'Species Plantarum', first edition, had included under the generic name *Convallaria* eight species. All but one, *C. majalis*, were later segregated and referred to *Polygonatum*, *Smilacina*, and *Maianthemum*. The assemblage of these plants in one genus by Linnaeus was based principally on the spotting of the young berries, a character which is more or less common to the entire group.

Desfontaines, considering several other characters, divided *Convallaria* into four distinct genera: *Convallaria*, *Polygonatum*, *Smilacina*, and *Maianthemum*. The distinguishing features were found in the nature of the flowers and in the manner in which they are borne on the stem. *Smilacina* was distinguished because of its terminal inflorescence, the division of the perianth into six separate segments, and the star-shaped corollas.

Five species of *Smilacina* were described by Desfontaines, two of which have been collected in Mexico, namely, S. *racemosa* (*Convallaria racemosa* L.), the type species of the genus, and S. *stellata*. Of the remainder, S. *ciliata* apparently is a synonym of S. *racemosa*, S. *trifolia*, a boreal species of both hemispheres which does not enter our range, and S. *umbellata*, which becomes *Clintonia umbellulata* Michx. Bertoloni[3] added a new species in 1840, S. *flexuosa*, and was followed two years later by Martens and Galeotti[4], who described three additional species and one variety: S. *macrophylla*, S. *scilloidea*, S. *paniculata*, and S. *scilloidea* var. *acutifolia*. *Smilacina amoena* was published by Wendland[5] in 1850.

J. G. Baker[6] was the first to compose a monograph of this group, but used the earlier name *Tovaria* Necker. In this work four new species appeared: *T. thyrsoidea*, *T. laxiflora*, *T. nervulosa*, and *T. Salvini*. The first three species are regarded in this revision as synonyms of S. *paniculata* Mart. & Gal., whilst the

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[1] Desf. Ann. Mus. Par. **9**:51, *t. 9*. 1807.
[2] Linn. Sp. Pl. ed. 1. 315. 1753.
[3] Bertol. in Nov. Comm. Acad. Bonon. 4:411. *pl. 39*. 1840.
[4] Mart. & Gal. in Bull. Acad. Brux. **9**[1]:387–388. 1842.
[5] Wendl. in Otto & Dietr. Allg. Gart. Zeit. **17**:137. 1850.
[6] Baker in Jour. Linn. Soc. Bot. **14**:564. 1876.

* An investigation carried out at the Missouri Botanical Garden and submitted as a thesis in partial fulfillment of the requirements for the degree of master of science in the Henry Shaw School of Botany of Washington University.

Issued November 30, 1945.

fourth apparently is a color variety of S. *amoena* Wendl.

In his treatment of *Smilacina* for the Botany of the 'Biologia Centrali-Americana,' Hemsley[7] merely abstracted the revision of Baker, and making the necessary transfers from *Tovaria* recognized seven species: S. *flexuosa*, S. *laxiflora*, S. *nervulosa*, S. *paniculata*, S. *Salvini*, S. *scilloidea*, and S. *thyrsoidea*. Like Baker, Hemsley was unable to place S. *paniculata* satisfactorily, considering it as identical with S. *amoena*, and thought S. *macrophylla* to be a synonym of S. *scilloidea*.

Since 1884 the only new species of *Smilacina* to be published from Central America and Mexico is S. *Gigas* Woodson[8], from Panama, which probably is only a giant phase of S. *paniculata* Mart. & Gal.

Because of its popularity and general use, the generic name *Smilacina* was conserved by the International Botanical Congress of Brussels (1910), since it is antedated by three previous genera: *Vagnera* Adans.[9], *Tovaria* Neck.[10], and *Polygonastrum* Moench[11]. Later generic synonyms include *Sigillaria* Raf.[12], *Stylandra* Raf.[13], *Asteranthemum* Kunth[14], *Jocaste* Kunth[15], *Medora* Kunth[16], and *Neolexis* Salisb.[17], all of which fall readily into synonymy.

II. Morphology and Generic Relationships

Roots.—The roots of *Smilacina* are borne either at the nodes or all over the surface of the underground rhizome. They are either simple or shortly branched, closely placed to one another and forming a dense mass as in S. *amoena* var. *Salvini*, or more loosely clustered at the nodes as in S. *scilloidea*. They are relatively slender and sometimes very long, measuring a foot or more in length. They are usually covered with a dense felt of persistent root hairs.

Rhizome.—The rhizome varies considerably both in size and in some external characters such as type of branching. But due to the difficulty in pressing these large underground stems, they are seldom collected and not much is known of their variability or taxonomic importance. They are essentially simple or branched, and apparently reach relatively great lengths in most species. They may be thick and very fleshy as in S. *amoena* var. *Salvini*, or quite slender as in S. *scilloidea*. The rhizome of S. *racemosa* is very knotty due to the closely budding upright shoots, while those of S. *scilloidea* and S. *stellata* are rather smooth and slender, since there is less budding.

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[7] Hemsl. Biol. Centr.-Am. Bot. **3**:367–368. 1884.
[8] Woodson in Ann. Mo. Bot. Gard. **27**:270. 1940.
[9] Adans. Fam. Pl. **2**:496. 1763.
[10] Neck. Elem. **2**:190. 1790.
[11] Moench, Meth. 637. 1794.
[12] Raf. in Jour. Phys. **89**:261. 1819.
[13] Raf., loc. cit. 102. 1819.
[14] Kunth, Enum. Pl. **5**:151. 1850.
[15] Kunth, loc. cit. 154. 1850.
[16] Kunth, loc. cit. 155. 1850.
[17] Salisb. Gen. Pl. Fragm. 64. 1866.

Stems.—The stems are erect, unbranched, and typically herbaceous, arising from the terminal bud of the rhizome. The basal portion usually is clothed with very thin, scale-like, sheath leaves from the terminal bud of the rhizome. The height of the stem varies from 0.2 to 3 m., and may be relatively constant as in S. *amoena*, S. *racemosa* and most of the other species, or extremely variable, as in S. *paniculata*. In the living state the color varies from green to somewhat reddish or purplish at the base or in the inflorescence.

Leaves.—The leaves are alternate, and may be either sessile or petiolate. They vary in shape from narrowly lanceolate to broadly elliptic. In length they measure from about 3 to 30 cm. with the same extremes in width. All have longitudinal primary veins, some of which branch from near the leaf-base and are more prominent than the others. The number of these principal veins is rather constant for certain species, but is often difficult to observe. The great majority of the species bear glabrous leaves, but in some plants of S. *flexuosa*, and generally in S. *stellata*, there is an inconspicuous pubescence. The leaf characters vary considerably in the genus, and often even in the same species. It would be difficult to delimit the species by using such characters because of their inconsistency amongst individuals clearly conspecific in other details.

Inflorescence.—The inflorescence of *Smilacina* is terminal, or very rarely with auxilliary inflorescences in the axils of the uppermost leaves. Bracts usually are apparent only at the lower nodes. The best characters for the distinction of the species are found in the form of the inflorescence. These characters are shown in the diagrams of fig. 1, which also is a good indication of the probable phylogeny within the genus. There apparently is an evolutionary series from a diffuse panicle to a typical raceme, resulting through the suppression of secondary peduncles.

In S. *paniculata* and S. *racemosa* the inflorescence is a typical panicle, the secondary peduncles being extensive and many-flowered. The pedicels are relatively short, scarcely longer than the perianth and occasionally even somewhat shorter. In S. *amoena* the paniculate structure is less obvious, and the secondary peduncles have become shortened and few-flowered; and the pedicels are much longer than the perianth.

The secondary peduncles have become completely reduced, or rarely only slightly manifest in S. *flexuosa*, S. *scilloidea*, and S. *macrophylla*. The chief indication of a previous compound nature is found in the clustering of the flowers at the nodes of the primary peduncle. In S. *flexuosa* the rachis is conspicuously flexuous or geniculate, and the flowers are borne in groups of twos, threes, or occasionally fours. In this species the pedicels elongate very conspicuously after anthesis. The rachis is essentially straight in S. *scilloidea*, and is scarcely stouter than the pedicels, which occur usually in twos. In S. *macrophylla* the inflorescence

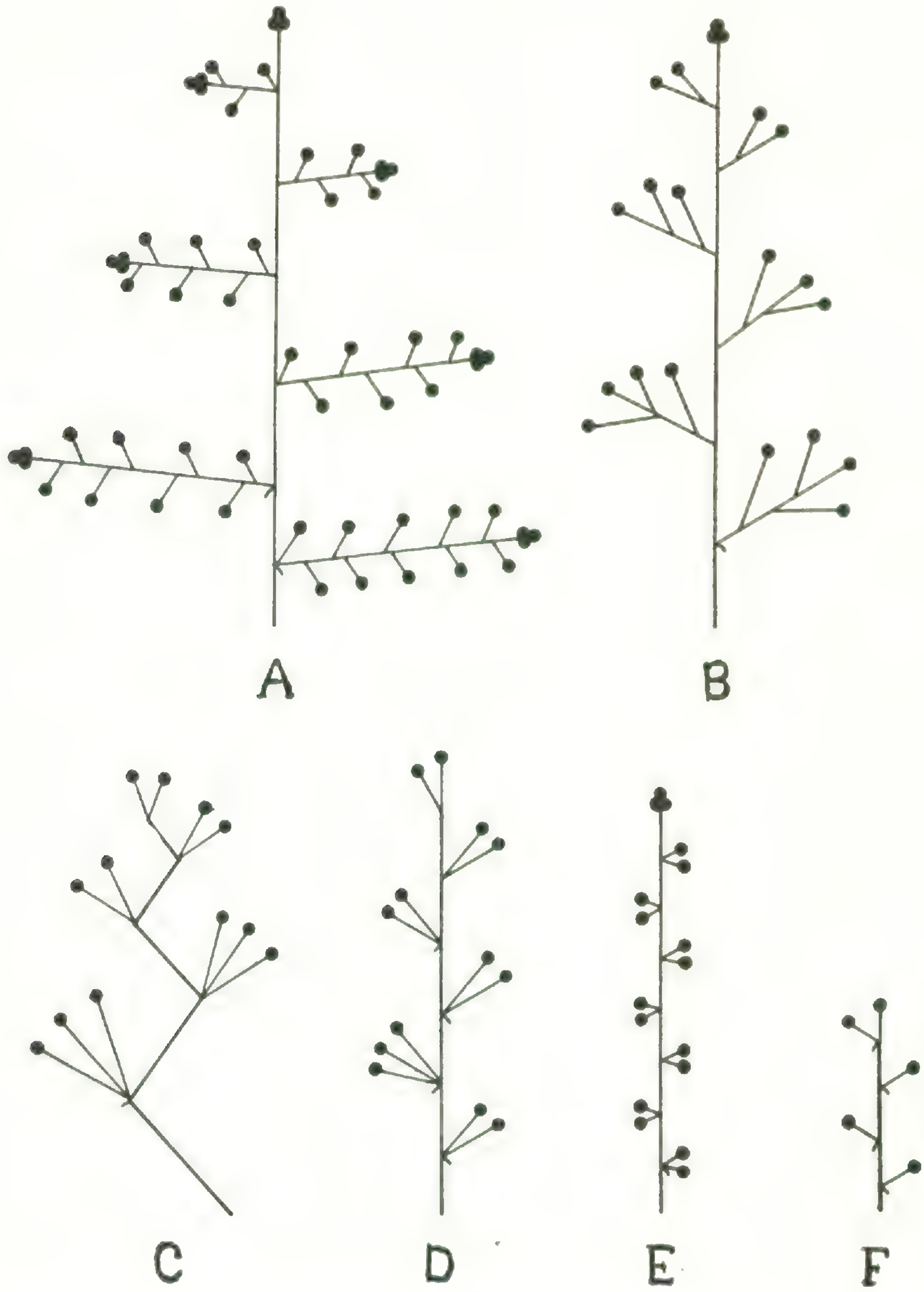

Figure 1. Inflorescence structures in *Smilacina:* A, S. *paniculata;* B, S. *amoena,* C, S. *flexuosa;* D, S. *scilloidea;* E, S. *macrophylla,* F, S. *stellata.*

is subspiciform, with a straight, stout rachis bearing short, horizontal or reflexed, paired pedicels.

The reduction of the inflorescence to a typical raceme is attained in S. *stellata*, where the pedicels are borne singly at the nodes.

Perianth.—In *Smilacina*, as in most Liliaceae, there is no distinction of calyx

and corolla except in the separation of the six segments into an outer and an alternating inner whorl. The outer and inner segments are exactly alike in shape and size, varying amongst the different species from lanceolate to broadly ovate in outline, and in length from 1 mm. *(S. racemosa)* to 9 mm. *(S. flexuosa)*. They may be widely spreading at anthesis, as in S. *paniculata*, or nearly erect and overlapping, as in S. *amoena* and S. *macrophylla*. The former condition is by far the more common. The perianth of *Smilacina* predominantly is white, but three closely related species, S. *flexuosa*, S. *scilloidea*, and S. *amoena*, include varieties with pink or flesh-colored flowers *(S. flexuosa* var. *rosea*, S. *scilloidea* var. *erubescens*, and S. *amoena* var. *Salvini)*. This parallelism in color variation is very suggestive of close relationship amongst the three species.

Stamens.—There are six stamens in flowers of *Smilacina*, and these are attached to the base of the perianth segments. The anthers are introrse, 4-celled, and split longitudinally at anthesis. They vary from 0.5 to 3.0 mm. in length, and are commonly yellowish, but sometimes blue, at least in S. *paniculata*. The filaments may be narrowly filiform or somewhat enlarged at the base, varying from about 1 mm. in length in S. *racemosa* to 4 mm. in S. *flexuosa*.

Pistil.—The pistil is typically liliaceous, being 3-carpellate and 3-celled. The style may be somewhat shorter or somewhat longer than the ovary, and is capped by the stigma which is more or less deeply 3-lobed. *Smilacina* previously has been described as having two ovules within each loculus of the ovary, but during this investigation it has been found that the number varies from 1, in some flowers of S. *flexuosa* and S. *paniculata*, to 5 or 6 in S. *amoena*. Although the number of ovules is not a constant character for the species, there is a general tendency toward a constant number in each species.

Fruit.—The fruit is a one- to several-seeded berry, more or less deeply 3-lobed. It is some shade of red or dark purple at maturity, rather spotted when young.

The closest relatives of *Smilacina* in North America are the genera *Convallaria* L., *Maianthemum* Desf., *Clintonia* Raf., and *Polygonatum* Desf. These may be distinguished as follows:

a. Perianth segments free.
  b. Stems leafy; leaves cauline, alternate; inflorescence paniculate to racemose.
    c. Flowers hexamerous ..... SMILACINA Desf.
    cc. Flowers tetramerous ..... MAIANTHEMUM Desf.
  bb. Stems scapiform; leaves basal; inflorescence umbellate ..... CLINTONIA Raf.
aa. Perianth segments coherent.
  b. Stems leafy; cauline leaves alternate; inflorescence axillary ..... POLYGONATUM Desf.
  bb. Stems scapiform; leaves basal; inflorescence terminal ..... CONVALLARIA L.

III. GEOGRAPHIC DISTRIBUTION

Smilacina is an outstanding example of the numerous genera having a common distribution in eastern Asia and eastern North America, to which Asa Gray called attention many years ago. In Asia the genus extends from Siberia to Japan and the Himalaya Mountains, represented by perhaps a dozen species. In North America its distribution ranges from Newfoundland to Alaska, southward to Panama.

In Mexico and Central America the genus is found only in the mountains at elevations of 1300–3300 m., usually in association with *Quercus*, *Alnus*, and other northern plant types. In such associations the plants of *Smilacina* frequently are common and terrestrial, but also grow on old stumps and mossy tree trunks from seeds probably deposited by birds after eating the pulpy berries.

Smilacina racemosa and S. *stellata*, which occur widely in the United States and northward, are found in Mexico only in the Sierra Madre of Chihuahua. But the majority of species have their center of distribution in Guatemala, extending northward to the Mexican States of Vera Cruz and Puebla and southward to the Province of Chiriquí, Panama. This distribution is that of S. *paniculata*, which is the most widespread of those in Mexico and Central America, as well as probably the most primitive of the species under discussion.

Smilacina amoena and S. *flexuosa* are nearly as widely distributed as S. *paniculata*, and occur throughout about the same territory from southeastern Mexico to Costa Rica. *Smilacina scilloidea* has been collected in Mexico, Honduras, and Guatemala, whilst S. *macrophylla* apparently is confined to southern Mexico. *Smilacina* apparently has not yet been found in Nicaragua, and only one doubtful specimen has been collected in Honduras. Future collections in those countries will surely show it to occur in the higher mountains, and help to explain some of the unsolved problems of this investigation.

IV. TAXONOMY

Smilacina Desf. in Ann. Mus. Paris **9**:51, *t. 9*. 1807; Endl. Gen. 1183. 1836-40; Benth. & Hook. Gen. Pl. **3**:770. 1883; Hemsl. Biol. Centr.-Am. Bot. **3**:367. 1884; Engl. in Engl. & Prantl, Nat. Pflanzenfam. ed. 1. **2**^{4}:79. 1888, *nomen conservandum*.

Vagnera Adans. Fam. Pl. **2**:496. 1763, *nomen rejiciendum*.
Tovaria Neck. Elem. **2**:190. 1790; J. G. Baker in Jour. Linn. Soc. Bot. **14**:564. 1876; Krause in Engl. & Prantl, Nat. Pflanzenfam. ed. 2. **15**^{a}:367. 1930, *nomen rejiciendum*.
Polygonastrum Moench, Meth. 637. 1794, *nomen rejiciendum*.
Sigillaria Raf. in Jour. Phys. **89**:261. 1819.
Stylandra Raf. loc. cit. 102. 1819.
Asteranthemum Kunth, Enum. Pl. **5**:151. 1850.
Jocaste Kunth, loc. cit. 154. 1850.
Medora Kunth, loc. cit. 155. 1850.
Neolexis Salisb. Gen. Pl. Fragm. 64. 1866.

Perianth homochlamydeous, equally 6-parted, white to pink or purplish. Stamens 6, epipetalous, the filaments filiform or dilated, the anthers introrse, 4-celled. Ovary superior, 3-carpellate, the loculi with 1–6 superposed anatropous ovules on an axile placenta, the style terminal, filiform, the stigma capitate or somewhat 3-lobed. Berries pulpy, usually red or purplish, 1–6-seeded. Herbs with simple stems from an extensive rhizome. Leaves alternate, shortly petiolate to sessile or somewhat amplexicaul, with 3 to many principal parallel veins and variable secondary and cross veins. Inflorescence usually terminal, occasionally in the upper leaf axils also, paniculate to racemose, bracts very small or lacking.

Type species: *Smilacina racemosa* (L.) Desf.

Specimens have been examined from five of the larger herbaria of the United States. These are abbreviated in the citation of exsiccatae as follows: Chicago Museum of Natural History (formerly Field Museum) (FM); Gray Herbarium of Harvard University, Cambridge, Mass. (GH); Missouri Botanical Garden, St. Louis (MBG); University of California, Berkeley (UC); United States National Herbarium, Washington (US). I am very grateful to the curators of these herbaria for their courtesy and interest, and particularly to Dr. J. M. Greenman, Curator of the Missouri Botanical Garden Herbarium, under whom this investigation was carried on.

KEY TO THE SPECIES AND VARIETIES

a. Inflorescence paniculate, the lateral branches of the rachis manifest (but the upper ones much reduced in S. *amoena*).
  b. Inflorescence typically paniculate, the lateral branches of the rachis many-flowered; pedicels scarcely longer than the perianth.
    c. Perianth segments 3–5 mm. long; anthers included ..... *1. S. paniculata*
    cc. Perianth segments 1.0–1.5 mm. long; anthers exserted ..... *2. S. racemosa*
  bb. Inflorescence subracemiform, the lateral branches of the rachis corymbose, less manifest and few-flowered, the upper ones much reduced; pedicels much longer than the perianth.
    c. Flowers white ..... *3. S. amoena*
    cc. Flowers pink ..... *3a. S. amoena* var. *Salvini*

aa. Inflorescence subracemose, the lateral branches of the rachis not manifest, or only rarely so, the flowers paired or clustered at the nodes.
  b. Inflorescence racemiform, the rachis scarcely stouter than the pedicels, the latter usually horizontal or ascending; perianth segments spreading, oblong-lanceolate, 2–3 times as long as broad.
    c. Rachis flexuous or geniculate; pedicels longer than the perianth; principal veins of leaves usually 5–7.
      d. Flowers white ..... *4. S. flexuosa*
      dd. Flowers purplish pink ..... *4a. S. flexuosa* var. *erubescens*
    cc. Rachis straight; pedicels as long as the perianth or somewhat shorter; principal veins of leaves usually 3.
      d. Flowers white ..... *5. S. scilloidea*
      dd. Flowers pink ..... *5a. S. scilloidea* var. *rosea*
  bb. Inflorescence subspiciform, the rachis straight, much stouter than the pedicels, the latter descending; perianth segments nearly erect, broadly oval, almost as broad as long ..... *6. S. macrophylla*

aaa. Inflorescence typically racemose, the rachis unbranched, the flowers solitary in the axils of minute bracts ..... *7. S. stellata*

1. Smilacina paniculata Mart. & Gal. in Bull. Acad. Brux. 9²:388. 1842.

Tovaria thyrsoidea J. G. Baker in Jour. Linn. Soc. Bot. 14:568. 1876.
Tovaria laxiflora Baker, loc. cit. 569. 1876.
Tovaria nervulosa Baker, loc. cit. 1876.
Smilacina thyrsoidea (Baker) Hemsl. Biol. Centr.-Am. Bot. 3:368. 1884.
Smilacina laxiflora (Baker) Hemsl. loc. cit. 1884.
Smilacina nervulosa (Baker) Hemsl. loc. cit. 1884
Smilacina Gigas Woodson, Ann. Mo. Bot. Gard. 27:270. 1940.

Stems 0.4–3 m. high, somewhat flexuous, glabrous; leaves shortly petiolate, narrowly lanceolate to broadly ovate, acuminate, 6–30 cm. long, 1–12 cm. broad, longitudinal veins closely parallel and equal, or widely separated with 3–8 prominent veins and less conspicuous intermediate parallel veins, lateral veins more or less visible in dried plants; inflorescence typically paniculate, the secondary branches extensive and many-flowered, 3–50 cm. long, 2–25 cm. broad; pedicels solitary, horizontal or ascending, 1–10 mm. long; flowers white; perianth segments ovate-lanceolate, 3–5 mm. long, 1.5–2.5 mm. broad, spreading; stamens included, 2–4 mm. long, filaments somewhat enlarged at base, 1–3 mm. long, anthers 0.5–2.5 mm. long; ovary and style about equal in length, 1–5 ovules in each loculus, 1–2 ovules most common; fruit 1–5-seeded.

MEXICO: VERA CRUZ: Orizaba, *Botteri 138* (GH, US); in wet forest, in region of Orizaba, *Botteri 914* (US); in moist mountain forest, Cerro de Chocoman, Canton Córdoba, May 12, 1907, *Seler & Seler 5174* (US, GH); Acultzinco, May 1, 1937, *Matuda 1161* (US). PUEBLA: ledges of barranco below Honey Station, alt. 1525 m., May 6, 1904, *Pringle 8836* (UC, FM, GH, MBG, US). OAXACA: Teotalcingo, Petlapa, alt. 800–1400 m., *Galeotti 5485* (GH, UC, US); Petlapa, June, *Liebmann 14637* (FM). CHIAPAS: Cerro del Boquerón, Aug. 1913, *Purpus 7022* (UC).

GUATEMALA: ALTA VERAPAZ: on stumps and banks, Chama to Cobán, alt. 1075 m., Aug. 23, 1920, *Johnson 630* (US); forest near Cobán, alt. 1600 m., Sept. 1907, *Tuerckheim II 1962* (GH, US); Pansamalá, alt. 1125 m., May 1887, *Tuerckheim 231* (US). SAN MARCOS: hanging from tree at upper edge of potrero, Volcán Tajumulco, alt. 1300–1350 m., March 13, 1940, *Steyermark 37655* (FM). BAJA VERAPAZ: damp forest, mountain side north of divide north of Santa Rosa, alt. 1650 m., March 30, 1939, *Standley* 69927 (FM).

COSTA RICA: CARTAGO: Volcán Irazú, alt. 3050–3450 m., Dec. 1, 1937–Jan. 1, 1938, *Allen 690* (FM); in the oak forest on the upper slopes, El Volcán Irazú, Aug. 18, 1925, *Dodge 3417* (GH, US); south slope of Volcán Irazú near Finca Chilena, alt. 2700–2900 m., March 4, 1930, *Dodge & Thomas 8073* (GH, MBG); southern slope of western or main cone of Irazú, above alt. 3050 m., July 31, 1937, *Hatch 178* (FM); vicinity of the crater of the Volcán Irazú, Aug. 24, 1935, *Quiros 336* (FM); southern slope of Volcán de Irazú, March, 1924, *Standley 36029* (US); southern slope of Volcán de Turrialba, near the Finca del Volcán de Turrialba, alt. 2000–2400 m., Feb. 22, 1924, *Standley 35186* (US). SAN JOSÉ: on tree in wet forest, near Finca la Cima, above Los Lotes, north of El Copey, alt. 2100–2400 m., Dec. 21–22, 1925, *Standley 42735* (US); La Hondura, alt. 1300 m., Aug. 15, 1933, *Valerio 790* (FM).

PANAMA: CHIRIQUÍ: vicinity of "New Switzerland", central valley of Río Chiriquí Viejo, alt. 1800–2000 m., Jan. 6–14, 1939, *Allen 1302* (MBG); trail from Cerro Punta to headwaters of Río Caldera, alt. 2250–2500 m., Jan. 14, 1939, *Allen 1446* (MBG); rain forest, Bajo Chorro, alt. 1825 m., Jan. 6, 1938, *Davidson 53* (FM); Volcán de Chiriquí, Boquete District, alt. 2900 m., July 16, 1938, *Davidson 990* (FM); Volcán de Chiriquí, alt. 3300 m., Feb. 27, 1918, *Killip 360* (US); forest edge, vicinity El Potrero Camp, Chiriquí Volcano, alt. 2800–3000 m., March 10–13, 1911, *Pittier 3071* (US); on rotten stumps, Valley of the upper Río Chiriquí Viejo, vicinity of Monte Lirio, alt. 1300–

1900 m., June 27–July 13, 1935, *Seibert 184* (GH, MBG); vicinity of Casita Alta, Volcán de Chiriquí, alt. 1500–2000 m., June 28–July 2, 1938, *Woodson, Allen & Seibert 852* (MBG); Loma Larga to summit, Volcán de Chiriquí, alt. 2500–3380 m., July 4–6, 1938, *Woodson, Allen & Seibert 1034* (MBG); valley of the upper Río Chiriquí Viejo, March 18, 1938, *P. White 57a* (MBG); valley of the upper Río Chiriquí Viejo, Summer 1937, *P. White 75* (MBG); vicinity of Bajo Mona and Quebrada Chiquero, alt. 1500 m., July 18, 1940, *Woodson & Schery 512* (MBG); Casita Alta to Cerro Copete, alt. 2300–3300 m., July 10, 1940, *Woodson & Schery 339, 341, 342* (MBG).

This species is extremely variable in size, ranging from 0.4 to 3.0 m. tall. The leaves are proportionately variable also. The inflorescence may be small, measuring only 3 cm. long and 2 cm. broad, or it may be large and spreading, 50 cm. long and 25 cm. broad.

2. Smilacina racemosa (L.) Desf. in Ann. Mus. Par. **9**:51. 1807.

Convallaria racemosa L. Sp. Pl. 315. 1753.
Tovaria racemosa Neck. Elem. **3**:190. 1790.
Polygonastrum racemosum Moench, Meth. 637. 1794.
Maianthemum racemosum Link, Enum. **1**:343. 1821.
Unifolium racemosum Britton in Trans. N. Y. Acad. Sci. **8**:74. 1889.
Vagnera racemosa Morong in Bull. Torr. Bot. Club **20**:480. 1893.

Stems 1.8–7.8 dm. high, flexuous, glabrous or slightly pubescent; leaves sessile or shortly petiolate, lanceolate-ovate, acuminate, under leaf-surface inconspicuously pubescent, chiefly lower portion and petiole, 3–4 times as long as broad, 6–15 cm. long, 1.5–5.5 cm. broad, many major and minor longitudinally parallel veins, lateral veins hidden; inflorescence typically paniculate, lateral branches several-flowered, 2.5–10.5 cm. long, 0.5–2.5 cm. broad; pedicels solitary, usually ascending or horizontal, 0.5–2 mm. long; flowers white; perianth segments lanceolate or oblong, 1.0–1.5 mm. long, 0.5 mm. broad; stamens exserted, 2–3 mm. long, filaments enlarged at the base, 1.5–2.5 mm. long, anthers 0.5 mm. long; style shorter than the ovary, 2 ovules in each loculus; fruit 1–several-seeded.

MEXICO: CHIHUAHUA: near Colonia Garcia in the Sierra Madres, alt. 2285 m., June 3, 1899, *Townsend & Barber 5* (FM, MBG, US); Las Cuevas, June 30, 1892, *Hartmann 544* (GH, US) cold ledges, Sierra Madre, Oct. 3, 1887, *Pringle 1487* (GH); Sierra Madre, 1899, *Townsend & Barber 8* (US).

With so few specimens, it appears unprofitable to attempt the association of our Central American S. *racemosa* with either var. *typica* or var. *cylindrata* as segregated by Fernald (in Rhodora **40**:406-407. 1938). Amongst even our four specimens, extent of branching and general shape of the inflorescence are quite variable, suggesting the hopeless variability in S. *paniculata*. Evaluation of the reported geographical gradient in S. *racemosa* of the United States is without the scope of this discussion.

3. Smilacina amoena Wendl. in Otto & Dietr. Allg. Gart. Zeit. **17**:137. 1850.

Tovaria paniculata Baker, loc. cit. 568. 1876, as to description and specimens cited, not *Smilacina paniculata* Mart. & Gal.
Smilacina paniculata Mart. & Gal. acc. to Hemsl. Biol. Centr.-Am. Bot. **3**:368. 1884, excluding specimens cited.

Stems 1.5–8.0 dm. high, somewhat flexuous, glabrous; leaves sessile and amplexicaul or shortly petiolate, narrowly ovate to broadly ovate-acuminate, 5–20 cm. long, 2–8 cm. broad, glabrous, 4–12 prominent longitudinal veins, with numerous and less conspicuous parallel veins between, lateral veins visible in dried plants; inflorescence subracemiform, not flexuous, 5–30 cm. long, 1.5–10 cm. broad; secondary branches much reduced above, few-flowered; pedicels 4–30 mm. long, horizontal or ascending; flowers white; perianth segments narrowly elliptic-obovate, 5–6 mm. long, 2.5–3.0 mm. broad, somewhat spreading; stamens included, 4 mm. long, filaments less than 1 mm. broad at the base, 3 mm. long, anthers 1.0–1.5 mm. long; ovary and style about equal in length, 4–6 ovules in each loculus; mature fruit little known.

MEXICO: VERA CRUZ: Nogales, May 2, 1937, *Matuda 1154* (MBG, US). CHIAPAS: 1864–1870, *Ghiesbreght 708* (GH, MBG).

GUATEMALA: ZÁCAPA: cloud forest in ravine bordering Quebrada Alejandria, summit of Sierra de las Minas, vicinity of Finca Alejandria, alt. 2500 m., Oct. 13, 1939, *Steyermark 29875* (FM). CHIMALTENANGO: Calderas, Oct. 25, 1937, *Johnson 1109* (FM).

COSTA RICA: SAN JOSÉ: on tree in wet forest, near Finca la Cima, above Los Lotes, north of El Copey, alt. 2100–2400 m., Dec. 21–22, 1925, *Standley 43587* (US); high plateau, alt. 2000 m., Jan. 23, 1935, *Valerio 1075* (FM). ALAJUELA: San Ramón, April 21, 1929, *Brenes 6828* (FM).

3a. **Smilacina amoena** var. **Salvini** (Baker) Emons, n. comb.

Tovaria Salvini J. G. Baker in Jour. Linn. Soc. Bot. **14**:567. 1876.
Smilacina Salvini (Baker) Hemsl. Biol. Centr.-Am. Bot. **3**:368. 1884.

Essentially the same as *S. amoena* but with flowers pink; perianth segments broadly elliptic, 6 mm. long, 3.5–4.5 mm. broad, erect.

GUATEMALA: CHIMALTENANGO: Bosques de la Sierra "Santa Elena", Tecpam, alt. 2500 m., Jan. 1, 1932, *Salas 1430* (FM); Tecpam, alt. 2740 m., Feb. 5, 1937, *Johnson 633* (FM); Santa Elena, 1933, *Skutch 232* (US); place not recorded, Jan. 1892, *Shannon 439* (US); Chicoy, alt. 2500 m., March 1892, *Shannon 358* (US); Volcán Zunil, alt. 2500–3800 m., Jan. 22, 1940, *Steyermark 34685* (FM); above Tecpam, March 11, 1931, *Collins & Kempton 21* (US); Cupressus forest, Cerro de Tecpam, region of Santa Elena, alt. 2400–2700 m., Dec. 26, 1938, *Standley 61123* (FM). QUEZALTENANGO: Volcano of Santa María, alt. 2400–3510 m., Jan. 24, 1896, *Nelson 3700* (GH, US). SAN MARCOS: along Quebrada Canjula, between Sabinal and Canjula, Volcán Tacana, alt. 2200–2500 m., Feb. 18, 1940, *Steyermark 36024* (FM); between San Sebastián and top of ridge of Volcán Tajumulco, alt. 3800–4000 m., Feb. 16, 1940, *Steyermark 35903* (FM). HUEHUETENANGO: on mountain between Sija and Huehuetenango, alt. 3000 m., Feb. 21, 1938, *Walsh s. n.* (MBG).

4. **Smilacina flexuosa** Bertol. in Nov. Comm. Acad. Bonon. **4**:411. *pl. 39.* 1840; Hemsl. Biol. Centr.-Am. Bot. **3**:367. 1884.

Smilacina Bertolonii Kunth, Enum. **5**:151. 1850.
Tovaria flexuosa (Bertol.) Baker in Jour. Linn. Soc. Bot. **14**:567. 1876.
Convallaria flexuosa Druce, Rept. Bot. Exch. Club Brit. Isl. **3**:408. 1914.
Vagnera flexuosa Standl. in Jour. Wash. Acad. Sci. **15**:457. 1925.

Stems 0.3–9 dm. high, more or less straight, glabrous or pubescent; leaves subsessile to shortly petiolate, narrowly lanceolate to broadly ovate and shortly acuminate, 6–22 cm. long, 1.2–7 cm. broad, 5–7 prominent longitudinal veins,

intermediate ones less conspicuous, numerous short lateral veins; inflorescence racemiform, 5–30 cm. long, 2–6 cm. broad; rachis geniculate or flexuous; pedicels paired or clustered at the nodes, 0.7–3 cm. long, scarcely thinner than the rachis, horizontal or ascending; flowers white; perianth segments oblong-lanceolate, 5–9 mm. long, 2–2.5 mm. broad, spreading; stamens included, 3–4 mm. long, filaments slightly enlarged at the base, 2–3 mm. long, anthers about 1 mm. long; style somewhat longer than the ovary, stigma slightly lobed, 1–2 ovules in each loculus, usually 1; fruit usually 1–4-seeded.

MEXICO: CHIAPAS: Cerro del Boquerón, Aug., 1913, *Purpus 7022* (US); damp forests, mountains east of Fenix or Phoenix, date lacking, *Purpus 10621* (US); Cerro del Boquerón, June, 1914, *Purpus 7416* (FM, UC, US); Chicharras, alt. 3000–6000 ft., Feb. 6, 1896, *Nelson 3762* (US).

GUATEMALA: ALTA VERAPAZ: near Tactic, alt. about 1500 m., April 5, 1939, *Standley 70485* (FM); San Martín, July 1, 1938, *Johnston 1305* (FM). BAJA VERAPAZ: Taltic, alt. 1400 m., April, 1882, *Lehmann 1315* (US). HUEHUETENANGO: Concepción bei San Martín im Gebüsch, alt. 2000 m., June 21, 1896, *Seler & Seler 3168* (GH, US). SAN MARCOS: shaded moist ravine slopes, between San Rafael and Guatemala-Mexico line, alt. 2500–3000 m., Feb. 21, 1940, *Steyermark 36321* (FM); barrancos 6 miles south and west of town of Tajumulco, alt. 2300–2800 m., Feb. 26, 1940, *Steyermark 36618* (FM); along road above Barranco Eminencia, alt. about 2700 m., March 14, 1939, *Standley 68568* (FM). JALAPA: Volcán Jumay, north of Jalapa, alt. 1300–2200 m., Dec. 1, 1939, *Steyermark 32400* (FM). CHIMALTENANGO: Chichavac, alt. 2400–2700 m., Nov.-Dec., 1930, *Skutch 73* (US); Cerro de Tecpam, region of Santa Elena, alt. about 2700 m., Dec. 4, 1938, *Standley 58708* (FM); region of Las Calderas, alt. 1800–2100 m., Nov. 22, 1938, *Standley 57810* (FM). SACATEPEQUEZ: slopes of Volcán de Agua, above Santa María de Jesús, alt. 2250–3000 m., Feb. 11, 1939, *Standley 65134* (FM); Volcán Agua, alt. 9500 ft., Feb. 8, 1908, *Kellerman 7295* (FM). SANTA ROSA: Santa Rosa, alt. 840 m., June, 1892, *Heyde & Lux 3527* (US); Volcán de Agua, alt. 2800 m., June, 1892, *Shannon 3634* (US).

EL SALVADOR: Cerro de Apaneca, 1928, *Calderon 2417* (FM).

HONDURAS: in forest near summit of the range above El Achote, in cloud zone above the plains of Siguatepeque, alt. 1850 m., Aug. 1, 1936, *Yuncker, Dawson & Youse 6267* (FM).

COSTA RICA: CARTAGO: El Muneco, alt. 5000 ft., June 19, 1928, *Stork 2714* (FM).

4a. **Smilacina flexuosa** Bertol. var. **erubescens** Emons, n. var.[18]

Flowers pink; otherwise essentially the same as the species.

MEXICO: CHIAPAS: Cerro del Boquerón, June, 1914, *Purpus 7415* (FM, GH, UC); Volcán Tacana, Chiquihuite, March 27, 1939, *Matuda 2846* (FM).

GUATEMALA: GUATEMALA: locality lacking, 1939, *Aguilar 232* (FM). ZACÁTEPEQUEZ: Volcán de Agua, alt. 2800 m., *J. D. Smith 2175* (US). QUICHE: Nebaj, alt. 1930 m., April, 1890, *Heyde & Lux 4647* (GH, US). SANTA ROSA: Zamorora, alt. 1535 m., April, 1893, *Heyde & Lux 4652* (GH, US). SAN MARCOS: between Todos Santos and Finca El Porvenir, alt. 1300–3000 m., March 1, 1940, *Steyermark 36972* (FM, TYPE); San Martín, Oct. 9, 1938, *Johnston 1305* (FM).

5. **Smilacina scilloidea** Mart. & Gal. in Bull. Acad. Brux. 9[2]:388. 1842.

Smilacina scilloidea var. *acutifolia* Mart. & Gal. loc. cit. 1842.
Tovaria scilloidea [*scilloides*] (Mart. & Gal.) Baker in Jour. Linn. Soc. Bot. 14:567. 1875 (?), misspelling.

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[18] **Smilacina flexuosa** Bertol. var. **erubescens** Emons, var. nov., ab specie floribus roseis praecipue differt.

Stems 1.5–8 cm. high, flexuous, glabrous; leaves slightly sessile to petiolate, narrowly lanceolate to ovate, shortly acuminate, 3.5–10 cm. long, 1.0–4.5 cm. broad, usually with about 3 major longitudinal veins and numerous minor ones between, lateral veins evident in dried plants; inflorescence racemiform, 4–6 cm. long, 1.3–2.5 cm. broad; rachis straight, pedicels usually paired at the nodes, 3–6 mm. long, scarcely thinner than the rachis, horizontal or ascending; flowers white; perianth-segments oblong-lanceolate, usually 4–5 mm. long, rarely 6 mm., 1–2 mm. broad, spreading; stamens included, about 3 mm. long, filaments 1.5–2 mm. long, anthers 1 mm. or less long; style and ovary usually about equal length, style sometimes longer, stigma slightly 3-lobed, 1–2 ovules in each loculus, commonly 2; mature fruit several-seeded.

MEXICO: MICHOACÁN: vicinity of Morelia, Cerro Azul, alt. 2200 m., 1910, *Arsène 5766* (MBG, GH); vicinity of Morelia, Companario, alt. 2100 m., Dec. 1910, *Arsène 5803* (US). OAXACA: 2230–2500 m., *Galeotti 5483* (US); Cerro San Felipe, alt. 3000 m., May 22, 1898, *Conzatti & Gonzalez 704* (GH, US); rich canyons, Sierra de San Felipe, alt. 2230–2740 m., May 22, 1894, *Pringle 4647* (GH, MBG, UC, US); northwest slope of Mt. Zempoaltepec, alt. 2230–3045 m., July 10, 1894, *Nelson 667* (US); San Miguel, alt. 3000 m., May 1917, *Reko 3840* (US); Lachopa, June 1841, *Liebmann 14630* (FM). CHIAPAS: 1864-1870, *Ghiesbreght 707* (GH, MBG); Cerro del Boquerón, June 1914, *Purpus 7416* (GH, MBG); District of Temascaltepec, Nanchititla, Aug. 12, 1933, *Hinton 4520* (FM, MBG, US); Cajones, July 9, 1935, *Hinton 7053* (MBG, US); oak woods, Temascaltepec, Nov. 8, 1933, *Hinton 5078* (FM).

HONDURAS: COMAYAGUA: in the forest near the summit of the range above El Achote, in cloud zone above the plains of Siguatepeque, alt. 1850 m., Aug. 1, 1936, *Yuncker, Dawson & Youse 6257* (FM, MBG).

5a. Smilacina scilloidea Mart. & Gal. var. **rosea** Emons, n. var.[19]

Flowers pink; otherwise essentially the same as the species.

GUATEMALA: CHIMALTENANGO: in open pine forest with dense tussock grass, slopes of Volcán de Acatenango, above Las Calderas, alt. 2700–2900 m., Jan. 3, 1939, *Standley 61890* (FM). QUEZALTENANGO: pine-fir forest, Volcán Zunil, alt. 2500–3800 m., Jan. 22, 1940, *Steyermark 34720* (FM); Volcán Santa María, alt. 3260 m., July 27, 1934, *Skutch 869* (FM,TYPE, GH).

6. Smilacina macrophylla Mart. & Gal. in Bull. Acad. Brux. 9^2:387. 1842.

Stems 4.5–6.0 dm. high, somewhat flexuous, glabrous; leaves shortly petiolate or subsessile, ovate-lanceolate, acuminate, 12.5–22.0 cm. long, 2.5–9.0 cm. broad, with 5–10 prominent longitudinal veins and less conspicuous ones between, lateral veins visible in dried plants; inflorescence subspiciform, several-flowered, 12.5–22.0 cm. long, 1.2–2.0 cm. broad; peduncle straight, much stouter than the pedicels; pedicels solitary, paired or clustered, 3-5 mm. long, horizontal or descending; flowers white (?); perianth segments broadly oval, 6 mm. long, 3.0–3.5 mm. broad, nearly erect; stamens included, filaments enlarged at base, 3 mm. long, anthers 1.5 mm. long; ovary and style of equal length, 4 ovules to each loculus; mature fruit unknown.

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[19] **Smilacina scilloidea** Mart. & Gal. var. **rosea** Emons, var. nov., ab specie floribus roseis praecipue differt.

MEXICO: VERA CRUZ: barranco of Teoxolo, near Jalapa, alt. 3500 ft., May 22, 1899, *Pringle 7854* (GH, US); Huitamalco, June 1841, *Liebmann 14635* (US, FM).

I have not seen the type specimen of *S. macrophylla, Galeotti 5473,* but the plants cited above agree well with the original description.

7. Smilacina stellata (L.) Desf. in Ann. Mus. Par. **9**:52. 1807.

Convallaria stellata L. Sp. Pl. 316. 1753.
Tovaria stellata (L.) Neck. Elem. **3**:190. 1790, name only.
Maianthemum stellatum (L.) Link, Enum. **1**:343. 1821.
Asteranthemum vulgare Kunth, Enum. **5**:152. 1850.
Unifolium stellatum (L.) Greene in Bull. Torr. Bot. Club **15**:287. 1888.
Vagnera stellata (L.) Morong in Mem. Torr. Bot. Club **5**:114. 1894.

Stems 1.5–5.5 dm. high, somewhat flexuous, glabrous; leaves sessile, amplexicaul, narrowly eliptical-lanceolate, acuminate, 4–15 cm. long, 1.0–4.5 cm. broad, upper leaf-surface glabrous, lower surface pubescent, many parallel longitudinal veins more or less of equal prominence, lateral veins usually hidden; inflorescence typically racemose, 2.5–7.0 cm. long, 1.5–2.0 cm. broad; peduncles unbranched, more or less straight; pedicels solitary, 2–10 mm. long, ascending; flowers white; perianth segments narrowly eliptical-oblong, 5–7 mm. long, 1.5–2.5 mm. broad, spreading; stamens included, 4 mm. long, filaments somewhat enlarged at base, 3 mm. long, anthers 1 mm. or less long; ovary usually longer than style, 2 ovules to each loculus; mature fruit 1–2-seeded.

MEXICO: CHIHUAHUA: collected near Colonia Garcia in the Sierra Madres, alt. 2290 m., June 16, 1899, *Townsend & Barber 35* (FM, GH, MBG, UC, US). NUEVO LEÓN: in shelter of thickets below timberline, alt. 11,700 ft., Cerro Potosí, July 9, 1938, *Schneider 1033* (FM).

This species is chiefly one of the United States, and reaches only a limited portion of northern Mexico where it is found in the higher altitudes. It is easily distinguished by its pubescent, sessile leaves, and simple raceme.

Explanation of Plate

PLATE 18

Fig. 1. *Smilacina paniculata* Mart. & Gal. Specimen collected in Valley of the Upper Río Chiriquí Viejo, vicinity of Monte Lirio, Province of Chiriquí, Panama, *R. J. Seibert 184*, in the Herbarium of the Missouri Botanical Garden.

Fig. 2. *Smilacina amoena* Wendl. var. *Salvini* (Baker) Emons. Epiphyte collected along Quebrada Canjula, between Sabinal and Canjula, Volcán Tacana, alt. 2200–2500 m., Dept. San Marcos, Guatemala, *J. A. Steyermark 36024*, in the Herbarium of the Chicago (Field) Museum of Natural History.

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Explanation of Plate

PLATE 19

Fig. 1. *Smilacina flexuosa* Bertol. Specimen collected in Chicharras, Chiapas, Mexico, *E. W. Nelson 3762*, in the United States National Herbarium.

Fig. 2. *Smilacina macrophylla* Mart. & Gal. Specimen collected in barrancos of Teoxolo, Vera Cruz, Mexico, *C. G. Pringle 7854*, in the United States National Herbarium.

FMONS—CENTRAL AMERICAN SMILACINAS

THE *CLEMATIS FREMONTII* VAR. *RIEHLII* POPULATION IN THE OZARKS[1]

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INTRODUCTION

During the latter half of the nineteenth century, the Darwinian theory of evolution by natural selection inspired a vast amount of research which was largely directed toward tracing of phylogenies and demonstrating the adaptation of organisms to their environment. However, the theory has recently been somewhat out of fashion. Its abeyance was coincident with the rise after 1900 of the new science of genetics and its companion, modern nuclear cytology. Preoccupation with the new disciplines partly accounted for the neglect of evolutionary studies. But it was partly due to the fact that the new principles which were emerging, the particulate theory of inheritance and the DeVriesian mutation theory, seemed to contradict some of the premises of Darwinism.

To-day there is a resurgence of interest in evolutionary matters. It is apparent that Darwin's theory, in its essentials, still stands. Modern genetics throws immediate light on some points which were hidden to Darwin. Gene mutation, which has now been studied in the laboratory and in the field, is seen to be the source of the omnipresent variation which Darwin pointed out but did not explain. The particulate nature of inheritance, far from being contradictory to the theory of natural selection, has been shown by Fisher ('30) to be essential to evolutionary change. A mathematical theory has been constructed, largely by Wright (for bibliography and non-mathematical summary, see Dobzhansky, '41), which permits rates of change of gene frequency to be calculated from mutation rates, the selective advantage of one gene over another, and size of population, under various systems of mating. These changes in gene frequency, when integrated for the entire genotype of the organism and over its entire population, may be said to constitute the primary steps in evolution.

The most important generalizations which Wright has made from his mathematical studies are those relating to the effect of population size, or more accurately, of what he terms "population number," upon the rate and course of evolution. In a very large, freely interbreeding population, where the number of potential mates for each breeding individual is large in relation to mutation rates, selection is strongly operative. The genotypes of the organism will then tend to cluster closely about a peak in the surface of adaptive values. The organism will be well adapted to its environment, but its over-all variability will be somewhat

[1] This paper is a revision of a dissertation which was prepared in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

restricted. It will adapt itself to a secular change in the environment by moving to a new adaptive peak, but it will not be able to cross an adaptive valley to reach a conceivably higher peak. In a very small population, or in one which is divided into small isolated colonies, the range of variation will be restricted locally, though there may be considerable variation from one colony to another. The phenomenon of "genetic drift" will come into play. There will be a random loss and fixation of genes resulting from the errors of sampling of the gametes which reproduce each generation, largely without regard to the adaptive value of the genes involved. As a result, the fate of an organism which is too greatly restricted in numbers is extinction. Wright considers the most favorable condition for continuing evolution to be that of a large population broken up into numerous small colonies which are connected by occasional migration. Each colony will be free to explore the field of gene combinations without the restrictive effect of too rigid selection. Differentiation within the population will be largely non-adaptive, but some of the colonies will be expected to arrive at favorable genotypes or adaptive peaks, perhaps quite different from the original one about which the population centered. Such colonies will tend to increase in numbers and to bring the remainder of the population up to their genotype through migration. This combination of non-adaptive differentiation of partially isolated local groups with intergroup selection will permit evolutionary advance without a secular change in conditions.

Wright's theory has become an important part of modern evolutionary thought. Eventually it may have the same importance and validity in the field of evolution which the publications of J. Willard Gibbs have in chemical thermodynamics. However, it is merely a theory, and it is impossible at present to judge whether it adequately accounts for evolutionary changes which are known to take place. It urgently requires testing against facts from the field. The facts required for its examination, or the examination of any other theory which attempts to explain the mechanism of evolutionary change, are of many kinds. The beauty of Wright's theory is that it indicates clearly the kinds of information which are important. Detailed information is required about life histories of various organisms, particularly the details of reproduction. Data are required on the numbers of individuals, and on their pattern of distribution, both at present and over a span of years. The pattern of differentiation must be understood in detail. Detailed information about sources of evolutionary change such as mutation, hybridization, and chromosomal changes must be obtained. Furthermore, the data on all these points must be coordinated for individual organisms. Such a body of detailed and coordinated facts scarcely exists for any organism, but is of first importance in any discussion of evolution.

The present study of *Clematis Fremontii* var. *Riehlii* was undertaken with the object of working out a picture of the features of its population structure which are of evolutionary importance, and if possible of making an estimate of evolutionary trends within the population. The pattern of distribution has been worked

out in some detail. Biological factors such as method of pollination, seed dissemination, seed germination and longevity have been examined. Variation in flower and leaf characters has been studied. An attempt has been made to obtain quantitative data where possible, but many of the present conclusions are based on subjective judgment; the difficulties are many.

Clematis Fremontii var. *Riehlii* is a member of the section VIORNA, subsection INTEGRIFOLIAE of *Clematis* (Erickson, '43a). Besides the Eurasian *C. integrifolia*, which probably should be placed in the subsection, it includes four closely related species and one or two varieties. They are comparatively well-marked and uniform entities, contrasting with such polymorphic species as *C. Pitcheri*. All except *C. ochroleuca* are of restricted distribution, characteristically occurring on rocky barrens. *C. albicoma* and the recently proposed *C. albicoma* var. *coactilis* (Fernald, '43) occur on the Devonian shale barrens of the Appalachians of West Virginia and Virginia (Wherry, '30, '31). *C. viticaulis*, also a shale barren plant, has been collected at a single locality. *C. Fremontii* is a secondary species in the *Andropogon scoparius* habitat of the mixed prairie of north-central Kansas. There it is usually limited to the upper slopes above the brows of hills where there is an outcrop of Fort Hays Limestone or Smoky Hill Chalk (Albertson, '37, '42).

C. Fremontii var. *Riehlii* is restricted to an area of somewhat more than 400 sq. mi. in Jefferson County and portions of two adjacent counties in east-central Missouri. A distribution map and a discussion of the limits of its distribution have been published (Erickson, '43b). The plant is wholly restricted to glades, rocky barrens which occur on south- and west-facing slopes of otherwise wooded ridges. The glades occur on outcrops of the thin-bedded dolomite of several formations of the Canadian Series, particularly the Cotter and Powell. Their distribution follows the outcrop belt of these formations; glades and the similar bald knobs of south-central Missouri encircle the Ozark dome. On a smaller scale, their occurrence is determined by the presence of sufficient local relief in conjunction with the outcrop of thin-bedded dolomite. They are characterized by a thin soil cover, which is slightly acid and fairly high in organic matter, and by an extreme set of environmental conditions: saturation to the point of seepage in late fall and early spring, and desiccation during the summer months (Erickson, Brenner and Wraight, '42). The glade habitat appears to be an edaphic climax, rather than a stage in the succession to upland forest, or a product of a biotic influence, such as grazing by cattle. The red cedar, *Juniperus virginiana*, is the most characteristic tree associated with the glades; the glades can be recognized from a distance by the contrast which the dark green of the cedars offers to the surrounding broad-leaved forest. The red cedars occasionally form an open cover, but usually occur as scattered individuals and may even be absent. The dominant plant is clearly the bluestem, *Andropogon scoparius*, though there are other grasses, and several other species make a conspicuous seasonal show of flowers, such as *Leavenworthia uniflora*, *Houstonia angustifolia*, and particularly, *Rudbeckia missouriensis*. Many of the plants have xeromorphic characteristics. Flor-

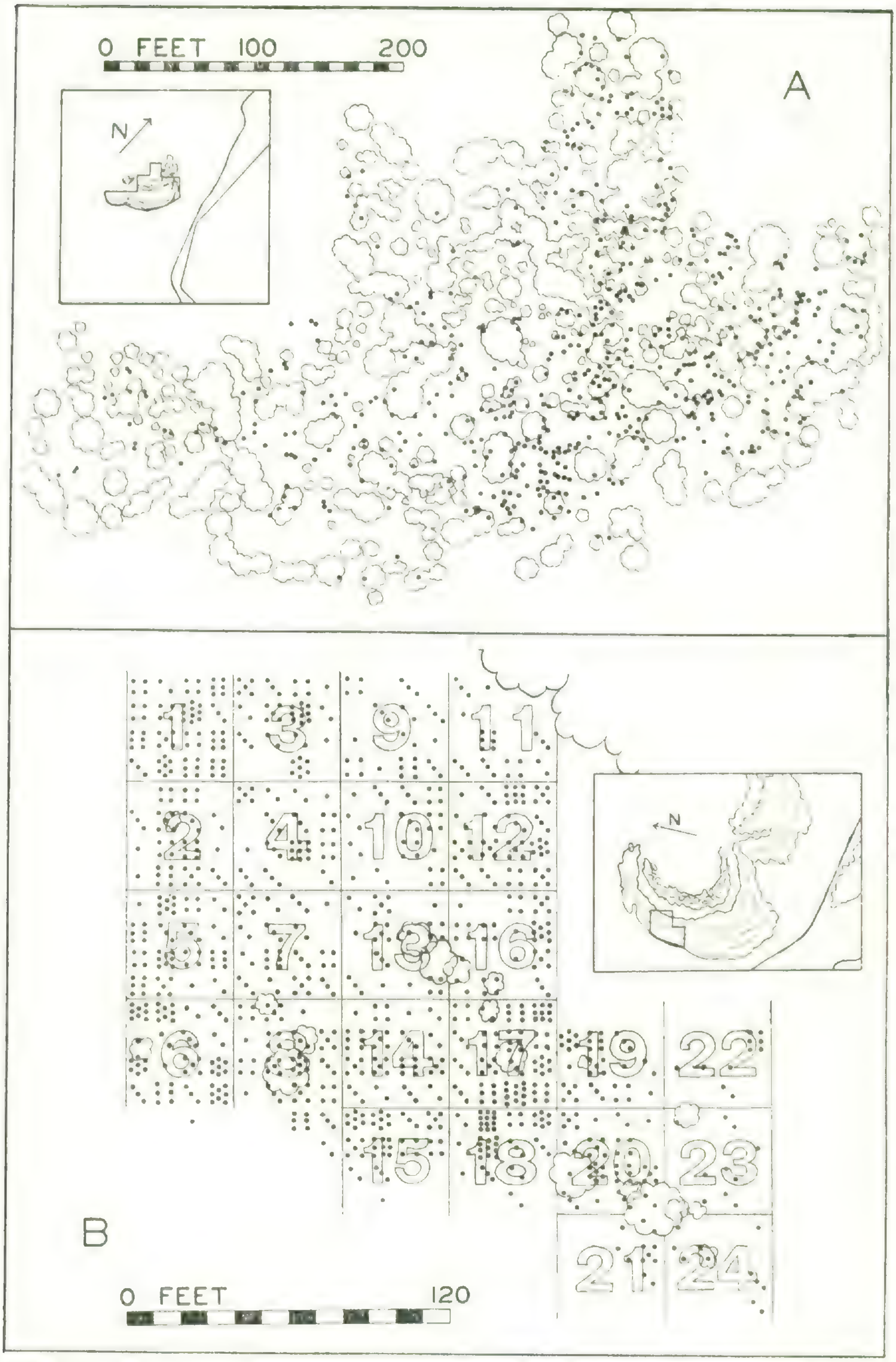

Fig. 1. Distribution of *C. Fremontii* var. *Riehlii* on greater part of small glade at R.2E, T.42N, S.10C (A); and on portion of larger glade at R.6E, T.39N, S.4-0 (B). Inserts show relation of area studied to glade as a whole. Small black dots represent *Clematis* plants, irregular outlines, trees. Domino effect in B is due to the fact that plants were counted in each 10-ft. quadrat, not plotted as they were for A. Numbered 40-ft. quadrats are referred to on p. 443 *et seq.*

istically, the glades are related to the shale barrens of the Appalachians (Wherry, '30), to the cedar glades of the Nashville Basin in Tennessee (Freeman, '33), to portions of the prairies of Kansas and Nebraska (Albertson, '37), and to glade-like grassy areas in the Arbuckle Mountains of Oklahoma and the Edwards Plateau of Texas.

Distribution Pattern

Because of its striking appearance, *Clematis Fremontii* var. *Riehlii* is a conspicuous member of the glade community, but in numbers it is subordinate. Its distribution on a number of glades has been studied in some detail. Figure 1 shows the distribution on the greater part of a small glade and a portion of a larger one that are not so much representative as illustrating approximately two extreme situations in which the plant is found. The maps were prepared from data obtained by laying out 10-ft. quadrats on the glades and plotting or counting the plants in each quadrat. On several other glades (fig. 2) the distribution has been studied by laying out 10-ft. transects of contiguous 10-ft. quadrats, usually at 250-ft. (50-pace) intervals, and normal to the "contour lines" formed by outcropping rock ledges.

Erickson and Stehn ('45) have published a statistical analysis of these data. They have pointed out that the data cannot be regarded as representing random (Poisson) distributions. Field observations suggested that the departure from randomness has its basis in a lack of uniformity of different portions of the glades as a habitat for *Clematis*. The data have been fitted by calculating two Poisson distributions for each glade, an "economic distribution," corresponding to suitable portions of the glade, and an "adventitious distribution," whose mean is small, representing unsuitable portions. The mean of the former is regarded as equivalent to Elton's ('32, '33) economic density.

The results of this statistical analysis should be considered in the light of field observations of conditions on the glades. The density counts are summarized in Table I. As contrasted with the tenfold variation in uncorrected, mean density, m_0, the economic densities, m_1, show a better agreement. The economic means of 1.02 and 1.10 plants per 100 sq. ft. are both for small glades; the rest, with means clustering around three or four plants per 100 sq. ft., apply to larger glades. The small glade at R.2E, T.42N, S.10C ($m_1 = 1.02$) is remarkable for its inaccessibility, and for the large size and number of red cedars. The data of line 6 in Table I ($m_1 = 1.10$) were obtained by combining data from two similar, adjacent glades in R.5E, T.40N, S.13. Both are small glades, though without such a conspicuous cover of red cedars as glade No. 1. The fact that the data appear to fall into two groups on the basis of economic density values is a reflection of the tendency, not recognized in the earlier field work, to select the larger, more "typical" glades for study. If more representative data were at hand, it would probably be found that the economic density is somewhat a function of the size of the glade, reaching an optimum value of three to four plants per 100 sq. ft. on large glades, and being smaller on smaller glades. It is thought that the conditions

TABLE I

DISTRIBUTION OF *CLEMATIS* ON GLADES IN FRANKLIN CO. AND JEFFERSON CO., MO.

(Glade numbers correspond with those of fig. 2)

No.	Location	Area (acres)	Est. number of *Clematis*	Mean density (m_0)	Economic density (m_1)
1	R. 2E, T. 42N, S. 10C	2.8	1,140	0.57	1.02
2	R. 2E, T. 42N, S. 15B	13.4	6,780	1.16	3.61
3	R. 3E, T. 41N, S. 25	23.3	3,880	0.41	2.98
4	R. 4E, T. 40N, S. 15B	14.9	5,580	0.86	3.87
5	R. 4E, T. 40N, S. 15D	14.8	13,230	2.05	4.07
6	R. 5E, T. 40N, S. 13A	5.4	830	0.34	1.10
7	R. 5E, T. 40N, S. 13E	20.7	32,000	3.69	2.51
8	R. 6E, T. 39N, S. 4-0	24.3	11,300	2.85	3.76

of winter saturation and summer desiccation, etc., referred to by Erickson, Brenner and Wraight ('42), are developed to the extreme only on the largest glades, being somewhat ameliorated on smaller glades more closely surrounded by forest. *Clematis* may be limited to glades not because of its special adaptation to their physical environment, but because it finds competition from other species too severe elsewhere, as Salisbury ('29) has found to be the case for other plants of barrens, such as *Ranunculus parviflorus*. If that is so, *Clematis* would be expected to reach its optimum density on the large glades where biological competition is presumably least severe.

The implication of this statistical treatment, that the glades can be divided into two portions on the basis of their suitability for *Clematis*, deserves some amplification. A prominent physical characteristic of the glades is the occurrence at intervals of parallel outcrops of more massive rock than the thin-bedded dolomite which forms the glade proper. On the aerial photographs of the region, which were studied as a preliminary to the field work, these outcrops give the appearance of contour lines, and aid greatly in recognition of the glades. In the field the ledges are found to vary greatly in distinctness. *Clematis* characteristically occurs just below such a ledge of rock, though it is by no means strictly limited to such places. This and the fact that it seems to be more abundant near the lower edge of a glade suggest that one of the factors determining its presence is the amount of seepage water available during the spring. The unsuitable portions of the glade, or "blanks," are of at least three kinds: the exceedingly barren areas just above a ledge of massive rock, which are strewn with chert fragments and occupied almost exclusively by a sparse growth of the small grass, *Sporobolus*

heterolepis; very grassy portions, where *Clematis* would presumably meet severe competition with *Andropogon;* and small clusters of trees, *Juniperus virginiana, Bumelia lanuginosa, Cornus florida,* etc., which occur at intervals on the glades, often where a gully has developed.

It is apparent then that the distribution of *C. Fremontii* var. *Riehlii* on individual glades is characterized by considerable aggregation. The *aggregates* of plants are not well delimited, as can be seen by reference to fig. 1, but they do exist. They vary considerably in area, and they may include a few plants to a few hundred.

While the aggregates of plants are undoubtedly important in breaking up the population into local groups, the *glades* themselves, by their greater definiteness of outline and more complete isolation, must also be significant. On the distribution map (Erickson, '43b, fig. 2), about 15 negative records were plotted within the distribution area of the *Clematis,* with 160-odd positive records, indicating that roughly 87 per cent of the glades support some plants. Furthermore, the plant has never been found except on a glade, and it is probably justifiable, as a first approximation, to regard glades and *colonies* of plants as equivalent in examining the organization of the population. Those which have been carefully studied (Table I) vary in area from 2.8 to 24.3 acres, and in estimated number of plants from 830 to 32,000. However, it has been pointed out above that the sampling involved has not been satisfactory. Between 200 and 250 glades have been visited more briefly, and some impressions gained from that experience should be pertinent. The glades vary in area from about 80 acres (large glade two miles north of Plattin) to small grassy areas which scarcely merit the name. In R.3E, T.41N, S.1–18, the total area in glades was measured by placing the tracings of the aerial photographs over a piece of paper ruled in small squares and counting the squares covered by glade outlines. Sixty glades were counted with a total area of 123 acres. Here, then, the average glade measures very nearly two acres in area. The number of plants per glade varies greatly, and probably corresponds only roughly with the area of the glade. Several glades of considerable area have been visited on which only one or a very few plants could be found. The upper limit in size of a colony is indicated by the figures in the fourth column of Table I, and the average size of a colony appears to be about 970, as calculated on page 422.

An impression of the degree of isolation between separate glades (= colonies) can be gained by examining fig. 2 and the larger scale map (fig. 3). On the whole, there is little difficulty in defining separate glades. It is apparent that the glades are not randomly distributed. No attempt has been made to treat this matter statistically, but obvious relations of the glades to the drainage pattern can be seen, as, for example, at R.4E, T.40N, S.11 and 14 (fig. 3) where the glades are ranged on either side of "branches" of Cotter Creek. Such topographically determined *clusters* of glades must also have significance in the subdivision of the population into local groups.

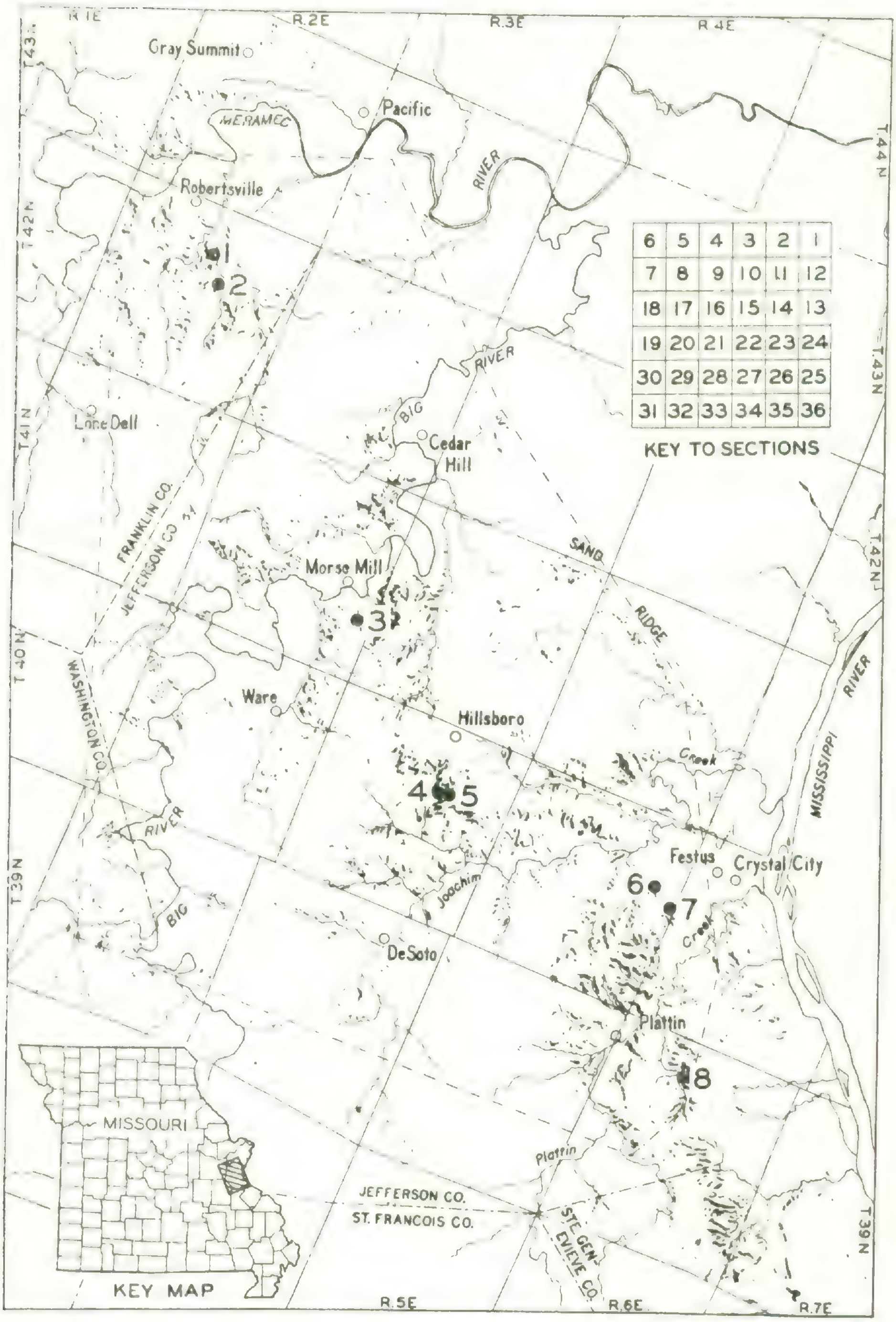

Fig. 2. Glades at which population density studies have been made. Glade numbers correspond with those of Table I.

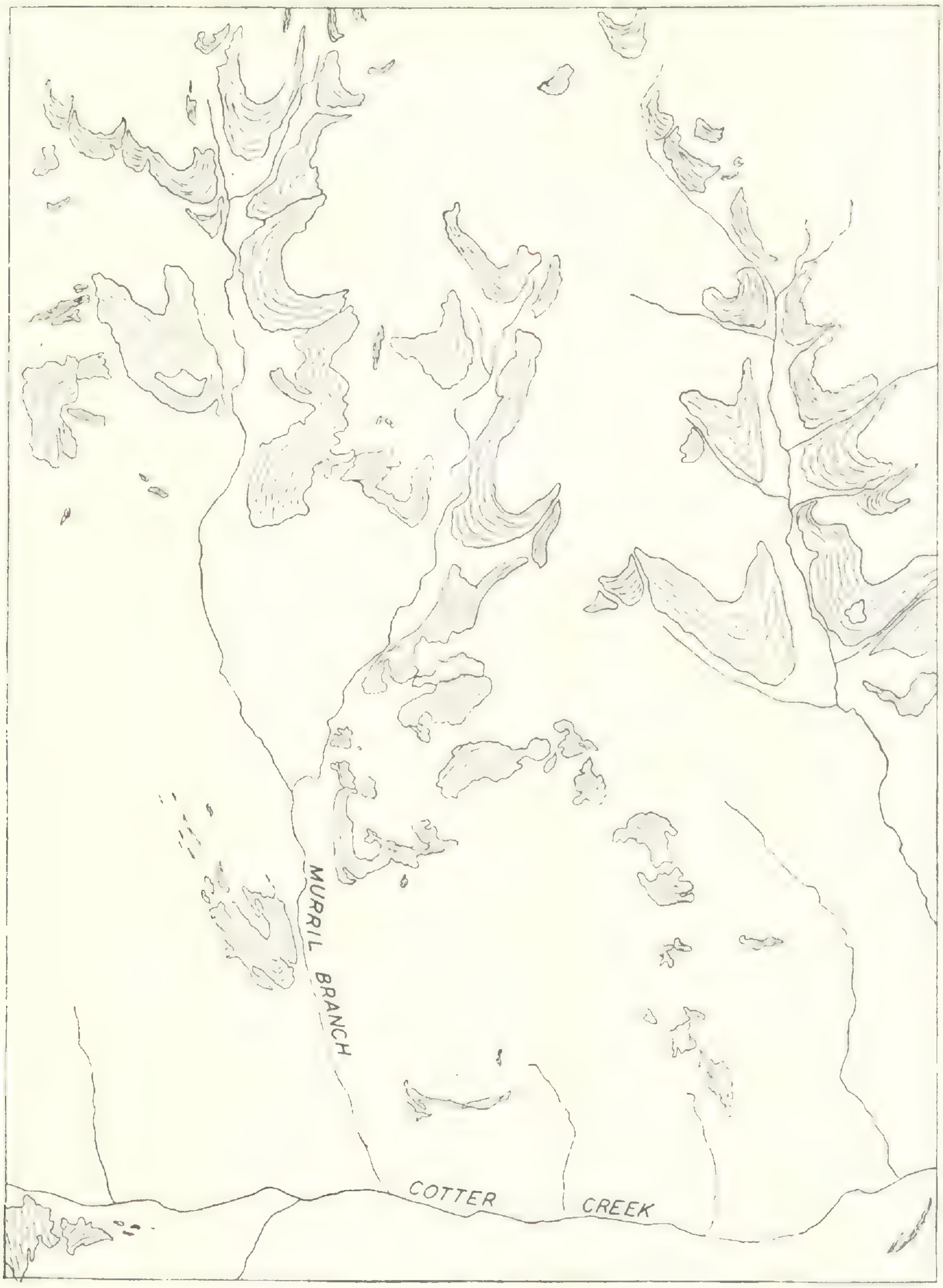

Fig. 3. Two clusters of glades in R.4E, T.40N, S.10, 11, 14, 15, 22 and 23, illustrating their relationship to the drainage pattern. Figure is a reduction of tracings of aerial photographs. Width of figure is two miles.

The next higher category of organization is seen in fig. 2 as a tendency for the entire distribution range to fall into four *regions* of glade concentration: (A) south of Robertsville, (B) about Morse Mill, (C) south of Hillsboro and (D) about Plattin. Scattered glades occur outside these regions of concentration. It is probable that the factors responsible for this large-scale grouping of the glades are variations in thickness of the determining strata of thin-bedded dolomite and the amount of local topographic relief. The four regious appear to be about equivalent in total glade area, but one has the impression from field work that the plant is most abundant on the glades about Plattin, and least abundant in the vicinity of Morse Mill, with the Robertsville and Hillsboro regions intermediate.

TABLE II

HIERARCHY OF SUBDIVISIONS OF THE *CLEMATIS* POPULATION

(Compare with fig. 4)

Subdivision	Number	Total area (sq. mi.)	Glade area		Number of *Clematis*
			(sq. mi.)	(acres)	
Distribution range	1	436	7.0	4,460	1,500,000
Regions	4	100	1.5	980	300,000
Clusters of glades	50	-----	0.09	60	30,000
Colonies (= glades)	1,450	-----	-----	2 (0.1–80)	970 (1–32,000)
Aggregates	15,000	-----	-----	0.2	97

It is thus seen that the distribution of *C. Fremontii* var. *Riehlii* falls naturally into a hierarchy of subdivisions, reminiscent of the hierarchy of subdivisions of the population of *Linanthus Parryae* which Wright ('43) devised for statistical reasons, but differing in that they have a natural basis and show no approach to equality in size. Some speculative calculations can be made of the relative size and number of the subdivisions (Table II). The estimate made in a previous paper (Erickson, '43b) of the total area over which *Clematis* is distributed stands; while several new records could now be added to the map, none are beyond the limits shown there. The calculations made in that paper of the total number of plants have been revised to include all the density data used in compiling Table I. The total was found to be 2,191,000, in gratifying agreement with the previously quoted estimate of 2,200,000 (rounded off from 2,197,000). However this figure has been reduced arbitrarily to 1,500,000, since the density counts weighted large glades too heavily. The estimate of the total number of glades, 1450, has been calculated by assuming that the 60 glades counted in the 18 sq. mi. at R.3E, T.41N, S.1–18, can be considered representative of the entire area. The average number of plants per glade, 970, has been obtained by dividing the number of glades into the total number of plants for the entire area. Similar calcula-

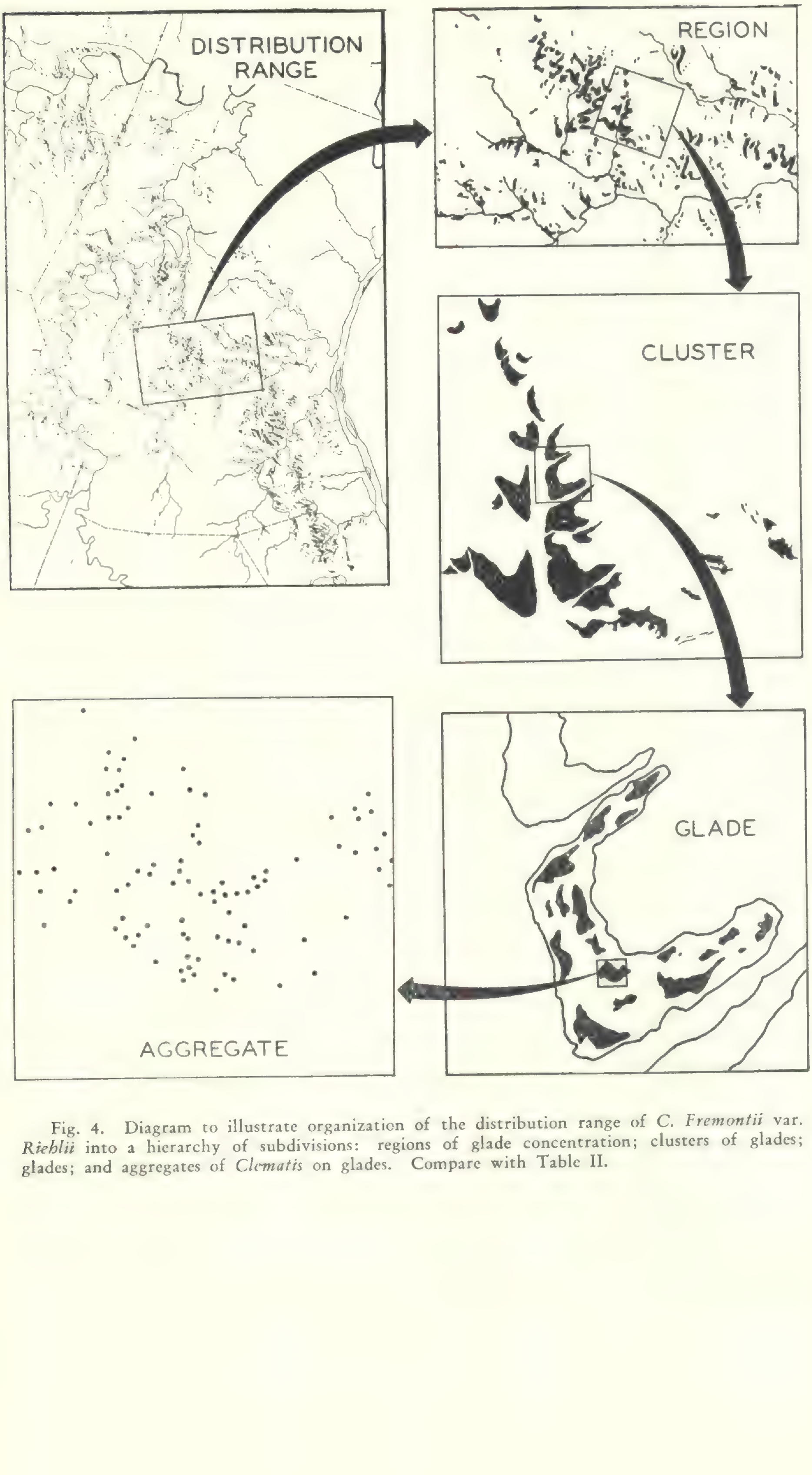

Fig. 4. Diagram to illustrate organization of the distribution range of *C. Fremontii* var. *Riehlii* into a hierarchy of subdivisions: regions of glade concentration; clusters of glades; glades; and aggregates of *Clematis* on glades. Compare with Table II.

tions, with liberal rounding-off of numbers, have given the other values in the table. An indication of the variation in numbers of plants has been given in parentheses for the glades. The other subdivisions also vary greatly in area and number of plants. The organization of the population into a hierarchy of subdivisions is illustrated diagrammatically in fig. 4.

On purely geographical grounds, then, *C. Fremontii* var. *Riehlii* can properly be described as a large population broken up into partially isolated groups. The partially isolated groups of greatest evolutionary significance are probably the aggregates of plants found to occur on each glade, difficult as they are to define in terms of area or number of plants. However, the concept of partial isolation applies equally well to the larger categories, the glades, the clusters of glades, and regions.

CONSTANCY OF NUMBERS

Great fluctuations in population size are known to occur in many organisms. Elton ('42, and other publications) has shown this to be the case for many northern mammals, and it is true of some species of *Drosophila* (see, for example, Spencer, '41). *Linanthus Parryae*, an annual plant which has been the subject of a population study, is reported to vary greatly in numbers from year to year (Epling and Dobzhansky, '42). Since the smallest size to which a population may be reduced largely determines its effective size for evolutionary purposes, the possibility of such fluctuations in this *Clematis* population must be considered. Albertson ('42) states that many plants of *C. Fremontii* in Kansas were killed during the years of drought from 1933 to 1939. The late drought, however, was not so severe in the Ozarks, which adjoin the Mississippi embayment, as it was on the prairies. This study was not begun long enough ago to have permitted any first-hand observations, but Anderson ('43) states that the drought of 1936 did not greatly harm many of the glade plants. Its main effect was to check *Andropogon scoparius*, so that other species which are normally held back by competition with it showed an unusually large display of flowers in the immediately following years. No specific observations of *C. Fremontii* var. *Riehlii* were made, but Anderson's opinion is that whatever damage it suffered during the drought was more than balanced by the release of competition from *Andropogon*. It might also be added that the habit of *Riehlii* of completing its growth by the middle of June probably contributes to its ability to withstand drought.

The influence of grazing on the numbers of *Clematis* is manifested in a similar way. The leaves, besides being very leathery when mature, are exceedingly acrid (Greshoff, '09, reports the presence of hydrocyanic acid in *C. Fremontii*), and cattle avoid them. The only evidence of disturbance by livestock is an occasional young shoot which has been nipped off when an inch or so above ground, presumably by error, and flowers which are occasionally removed without disturbance to the leaves. Grazing, however, keeps back the grasses, such as *Andropogon*, and the ultimate effect is to allow *Clematis* to increase both in numbers and in the

size of individual plants. This is strikingly seen in some cases where a fence divides a glade into a grazed and ungrazed portion. The plants on the grazed portion are noticeably larger, and flower somewhat earlier than those on the ungrazed part. Another biotic factor may be mentioned. The plant is subject to sporadic attacks by blister beetles, *Epicauta marginata,* which devour the leaves. Their attacks, however, are merely an annoyance to the investigator. They occur too late in the season, and are not frequent enough, to influence the population size of the plant seriously.

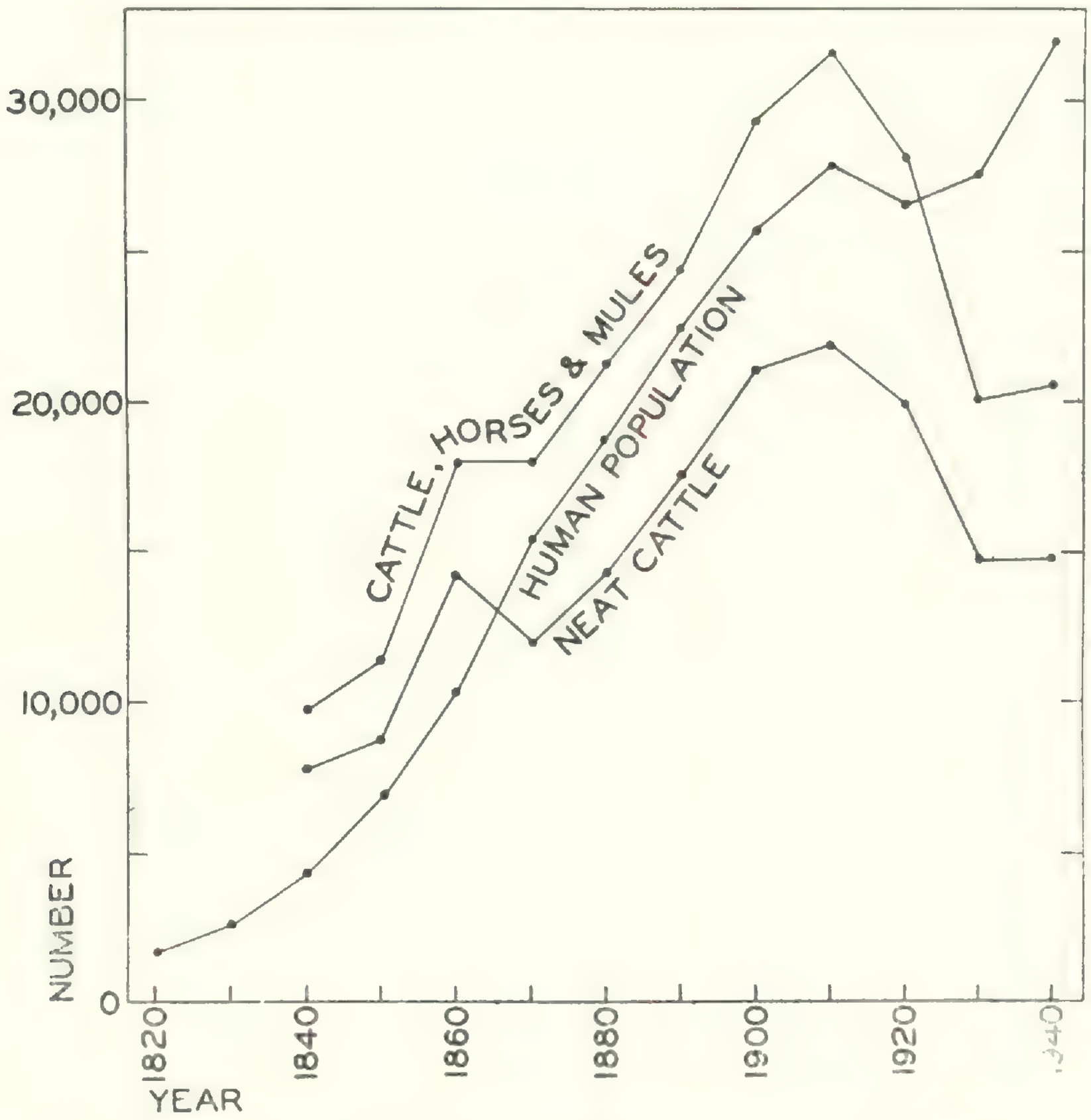

Fig. 5. Data on numbers of livestock and human population in Jefferson Co., Mo., from U. S. Census.

It is presumed that *Clematis* has increased in numbers since the white settlement of the country. United States Census data (fig. 5) show that the livestock population of Jefferson County reached a maximum in 1910, with a considerable decline until a minimum was reached in 1930, since when there has been an increase. Because the numbers of plants is believed to have varied roughly in proportion to the severity of grazing of the glades, it may be concluded that the population size of *Clematis* has increased considerably since 1800, and that it now

has reached relative stability, subject to fluctuations in relation to general economic conditions and changes in the management of individual farms. It is thought that the perennial habit of the plant may serve to damp such influences. Unfortunately, no direct evidence is at hand. A study of the old records of collection of the plant (Erickson, '43b) makes it seem probable that no conspicuous extension or restriction of range has occurred since the 1880's. Certainly no large-scale fluctuation in numbers has occurred in the four years during which the author has observed the plant. On the whole, the size of individual colonies appears remarkably stable as compared with the spectacular fluctuations which are known to occur in some other organisms.

The apparent constancy in size of this *Clematis* population at present does not, of course, imply that there have been no restrictions or extensions of its range in geological time. The presence of the very closely related *C. Fremontii* in Kansas suggests that it and *C. Fremontii* var. *Riehlii* must at one time have had a continuous distribution. A study of the distributions of other glade plants, particularly *Oenothera missouriensis*, suggests that the two *Clematis* populations may have been connected by way of the Edwards Plateau of Texas (unpublished maps prepared by Edgar Anderson). The separation into two populations may have occurred during the semi-arid period of late Pleistocene, or, in view of the importance of competition from grasses, during the warmer, moister period which followed (Sears, '35).

LIFE HISTORY

Clematis Fremontii var. *Riehlii* is a herbaceous perennial with a woody stem and remarkably coriaceous, prominently veined leaves, which have inspired the common name, "leatherleaf." It flowers during the last week of April and the first week of May, though it was seen flowering sporadically in September, 1941, a month of unusually high rainfall. Growth is completed within three weeks or a month after flowering, and the plants remain green for eight or ten weeks, turning brown during July. Because of their woody nature, many of the stems remain in place until February or March of the following year, the leaves by that time having become skeletonized and weathered to attractive gray laceworks of veins. However, some of the plants have been blown free of their moorings by October. A large plant forms a roughly spherical mass of rigid stems and leaves, and when it is freed, it may be carried for some distance over a glade as a tumbleweed.

The persisting structure is a woody caudex (fig. 6), provided with a mass of brown fleshy roots, in which the reserve food is starch. Two or four lateral buds are formed in the fall at the lower nodes of the old stem, one or more of which unfolds the next spring to form a new shoot. This process, over a period of years, gives rise to a certain amount of branching of the caudex, the older portions of which are torn apart by the growth of the roots. A large plant may consist of perhaps 20 shoots, arising from four or five separate caudices. Branching of the

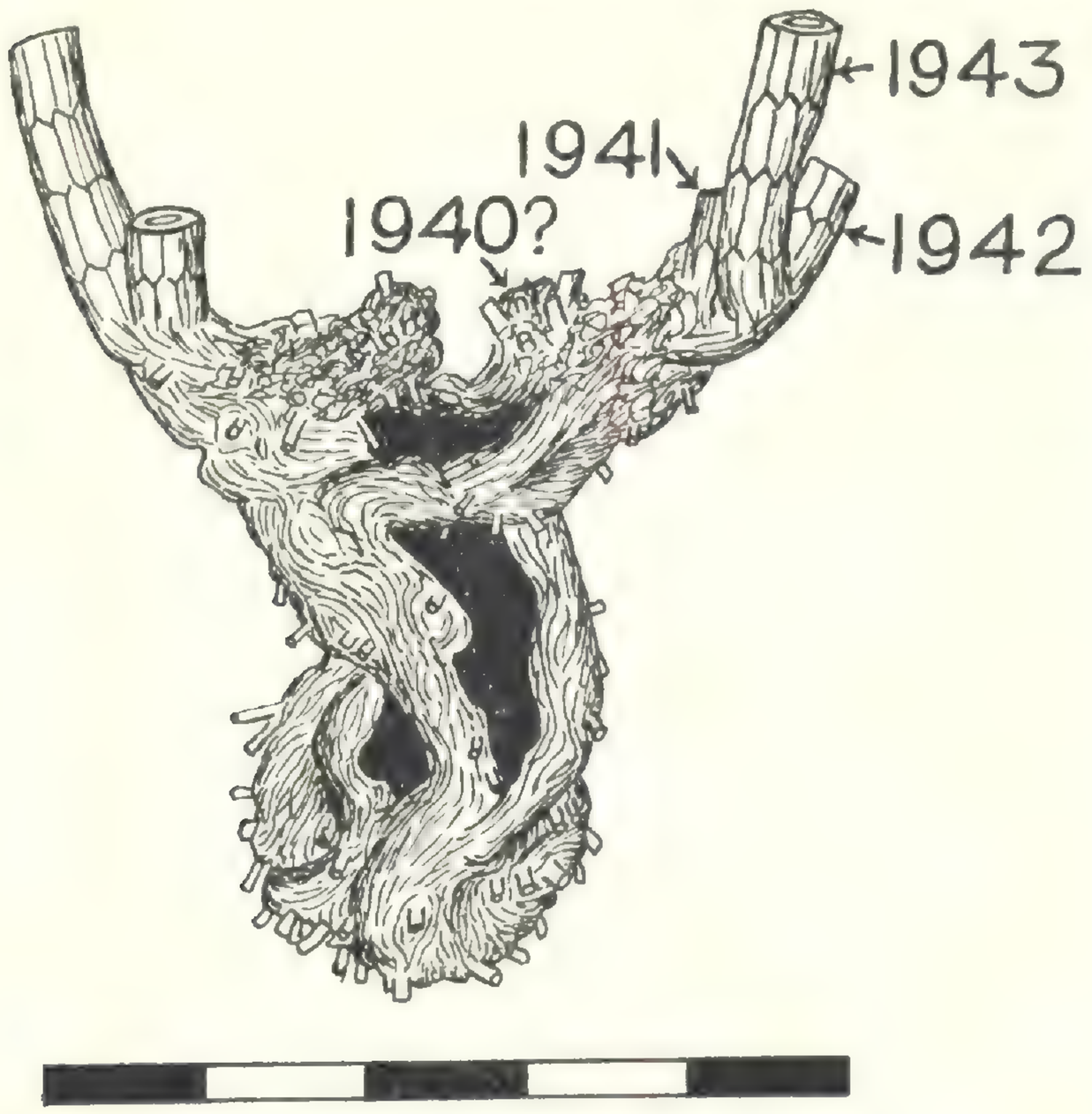

Fig. 6. Caudex of *C. Fremontii* var. *Riehlii*, which supported two shoots in 1943. It was collected in June, and lateral buds had not yet developed. Fleshy roots have been removed. Scale in centimeters.

caudex apparently does not provide a very efficient means of vegetative propagation. Morphological variation from plant to plant is sufficient to permit genetic individuals to be distinguished with some certainty. In a careful examination of perhaps 200 plants for evidences of clonal reproduction, only one case was found in which two separate plants appeared to belong to the same clone. They were about one foot apart, and there was evidence that the separation was accidental, caused by the fall of a tree trunk over the original clump. Three or four clumps were found which were actually two plants. In the population density studies reported above, each plant (or clump) old enough to have flowered has been scored as an individual regardless of its size or number of shoots.

In most species of *Clematis* the achenes are provided with conspicuous plumose tails, presumably well adapted to wind dispersal (fig. 7). In *C. Fremontii* var. *Riehlii*, however, the achene-tails are naked for the greater part of their length, though their basal portions and the apices of the achenes are silky (fig. 8). They are not suited for wind dispersal in the usual sense. Dispersal by the fur of a mammal is hard to visualize, and no evidence has been seen of their use as food by a bird or mammal. Some dissemination is probably achieved by the tumbleweed habit of the largest plants, but most of the achenes merely fall to the base of the parent plant. Dispersal of the achenes over a single glade is probably adequate, but the transportation of achenes from one glade to another must be a rare occurrence.

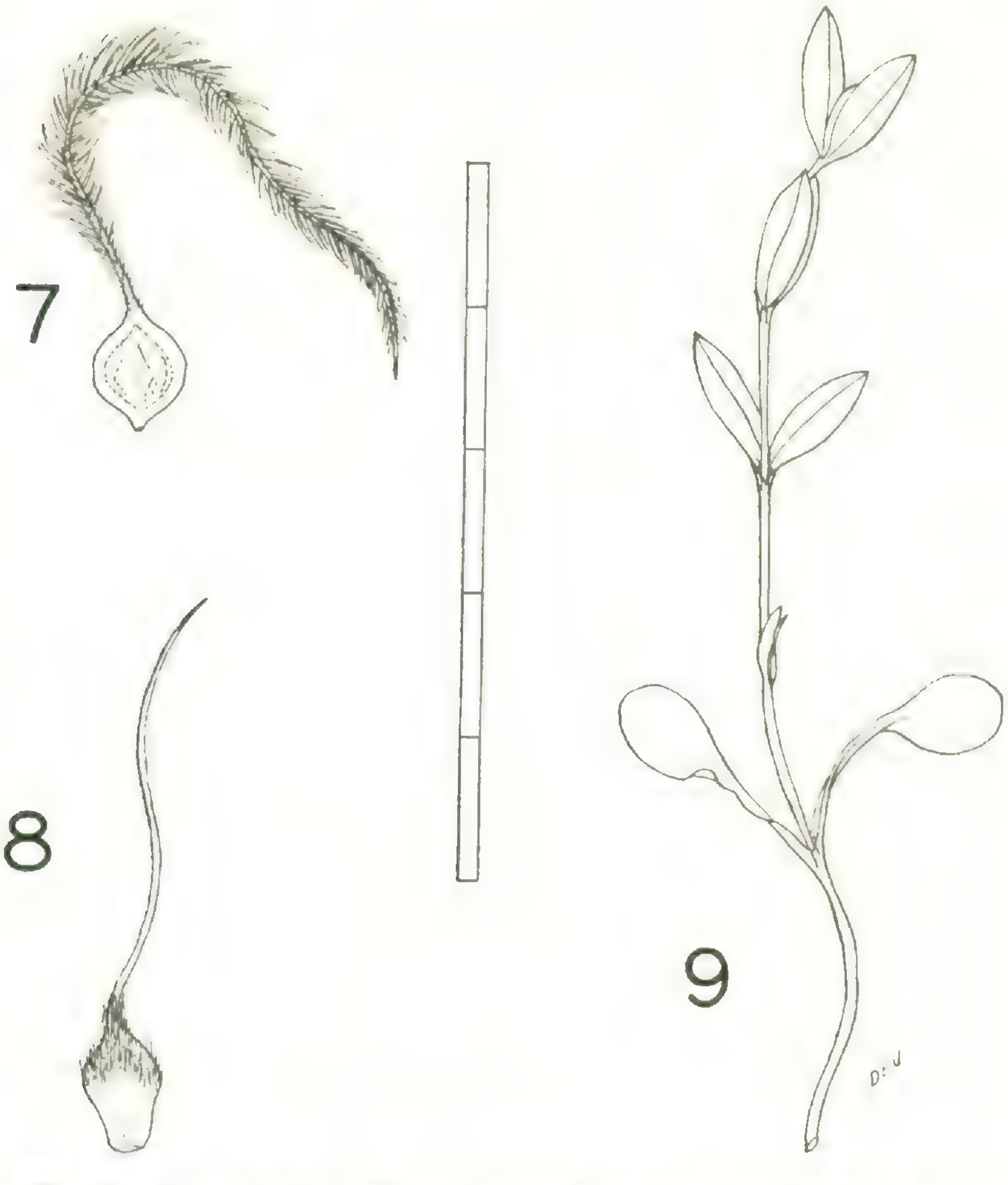

Fig. 7. Achene of *C. Viorna*, showing plumose achene-tail. Fig. 8. Achene of *C. Fremontii* var. *Riehlii*, showing naked achene-tail. Fig. 9. Seedling of *C. Fremontii* var. *Riehlii*, perhaps one month after germination. Scale in centimeters.

Attempts to germinate seeds under greenhouse conditions have been largely unsuccessful. Of about 600 seeds planted, three have germinated. Better success would perhaps be had by layering, but the experiments have not been carried out. Indications are that the percentage of germination of seed in the field is also low. The seedlings escaped detection in the early field work, because of their minute size (fig. 9). Many small sterile plants were found, but examination of the caudex always showed them to be two or more years old. When the seedlings were finally recognized, little trouble was had in finding them on any glade where careful search was made. They are not abundant. Presumably achenes are dropped in the vicinity of every large plant every fall; groups of seedlings can be found in such places in perhaps one case in 100. The conditions required for germination are not well understood, but adequate shade appears to be one of them. Germination takes place in the spring. Seedlings have not been found in September and October, though these months are often characterized by warm rainy weather, similar to that of April.

Growth of the plants seems to be quite slow, four or five years apparently being required from germination until the first flower is produced. Young, sterile plants (fig. 10) have been found on all the glades which have been studied. At R.4E, T.40N, S.15D, 61 of the 528 plants counted, or 8.7 per cent, were such plants. The conclusion that four or five years are required before flowering is based on examination of the caudices of many sterile plants, and of young plants which have produced a single flower. It is more difficult to estimate the age of larger clumps, since the older portions of a caudex are badly fragmented, and annual rings in the wood of the caudex are quite indefinite. Deduction from the size of the plant and of the caudex places the age of large clumps, such as illustrated in fig. 11, at 15 or 20 years, though there is no reason for thinking that they may not be indefinitely older.

Pollination

Since vegetative reproduction and seed dispersal are quite inefficient, pollen transportation must be looked to as the principal means of gene exchange from one glade to another, and perhaps also from one portion of a glade to another. The flowers are insect-pollinated. They are protogynous, as will be seen from fig. 12, and produce nectar at the base of the stamens. In view of these facts, cross-pollination would seem to be the rule, and more will be said about that below. However, the filaments elongate after the anthers have dehisced, and in an old flower the inner anthers are in contact with the style-tips, so that self-pollination is at least mechanically possible. Glassine bags have been placed over a number of flowers before anthesis to determine the seed-set in enforced self-pollination. The results have been nearly inconclusive. In the first attempts the bags were fastened around the peduncles of flowers, and with one exception failed to stay in place. When the bags were placed over several leaves as well as the flower, the plant and bag were blown over in the wind, became wet, and in most cases molded. Of 106 bags which were placed and later collected, three contained a full head of achenes, and three a few seeds each. The failures to set seed are attributed to the injury done the plant by enclosing it. Normally seeds are set by all the flowers except the smallest ones which occur late in the season on weak branches. Tentatively it is perhaps safe to assume that a plant will be self-pollinated if cross-pollination does not occur first.

Clematis is visited by a variety of insects while it is in flower. An insect net was carried for seven days during April, 1943, and as many as possible of the insects found on the flowers were captured. The specimens have been identified by Mr. Harold I. O'Byrne, and Mr. Richard Froeschner, with the exception of some smaller Hymenoptera. The data are presented in Table III. The most frequently found insects are four species of Pentatomidae. They are typically found lurking at the base of a flower, often with the proboscis inserted into one of the fleshy sepals. It is doubtful whether they are concerned in pollination, since they rarely venture to the opening of the flower, and apparently do not move from one plant to another often. The most conspicuous visitors, in order of the

TABLE III

INSECTS COLLECTED ON *CLEMATIS* FLOWERS

Order Family Species	Number of specimens		
	♂	♀	Total
Homoptera			
Cicadellidae			
Oncometopia lateralis (Fab.)		1	1
Hemiptera			
Pentatomidae			
Euschistus variolarius (Beauv.)	19	3	22
Eu. euschistoides (Voll.)	4	1	5
Thyanta custator (Fab.)	2	2	4
Peribalus limbolarius Stål.		1	1
Neididae			
Neides muticus (Say)	2	2	4
Lepidoptera			
Papilionidae			
Papilio ajax Linn.	1	1	2
P. troilus Linn.		2	2
P. philenor Linn.	1	1	2
Lycaenidae			
Strymon melinus Hbn.	1		1
Everes comyntas (Godt.)	1	1	2
Hesperiidae			
Proteides clarus (Cram.)	2	1	3
Thorybes pylades (Scud.)		1	1
Th. bathyllus (Ab. & Sm.)	3		3
Erynnis brizo (Bdv. & Lec.)	1		1
Sphingidae			
Hemaris diffinis (Bdv.) form *tenuis* Grote	5	6	11
Coleoptera			
Dermestidae			
Cryptorhophalum picicorne Lec.			1
Melyridae			
Collops vicarius Fall	1		1
Hymenoptera			
Bombidae			
Bombus impatiens Cresson		2	2
B. americanorum (Fab.)		3	3
Apidae			
Apis mellifica Linn.			1
Unidentified			
Hymenoptera (5 species ?)			12
Arachnida, Thomisidae			15

frequency with which they have been seen on the flowers, are the hawk moth, *Hemaris diffinis*, the bumblebees, *Bombus impatiens* and *B. americanorum*, and the swallowtails, *Papilio ajax*, *P. troillus* and *P. philenor*. They alone of the insects captured have proboscides long enough to reach the nectaries from the opening of the flower, a distance of about two cm. It is doubtful whether *Hemaris* or the *Papilio* species are involved in pollination to a considerable extent. The manner in which they cling to the recurved tips of the sepals while obtaining nectar suggests that they may be able to visit many flowers without picking up

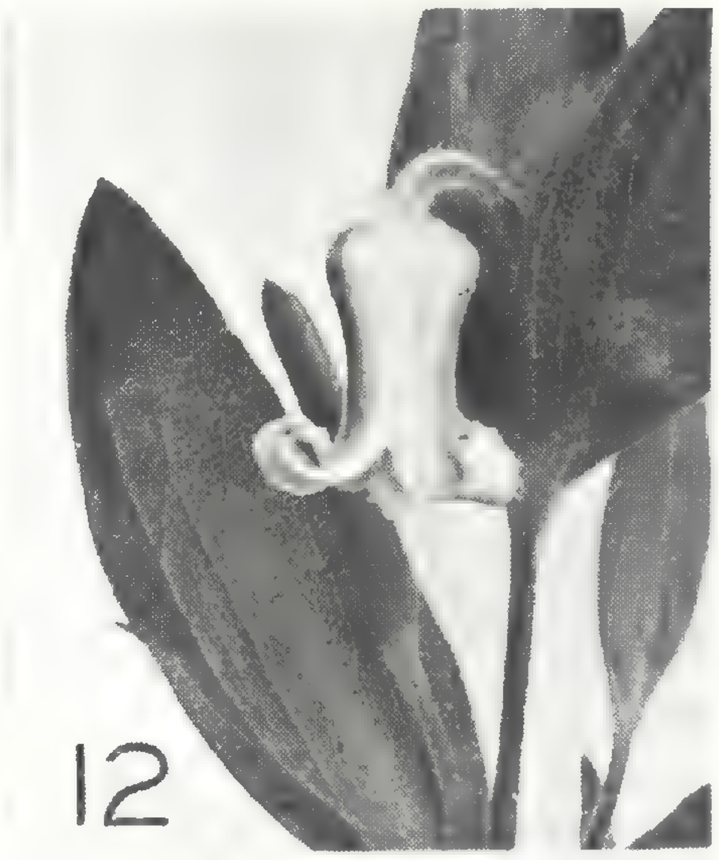

Fig. 10. Plant of *C. Fremontii* var. *Riehlii* estimated to be four years old. Scale in centimeters. Fig. 11. Mature plant of *C. Fremontii* var. *Riehlii* probably 15 years old or older. Scale in centimeters. Fig. 12. Flower of *C. Fremontii* var. *Riehlii*. Note that styles are exserted. Anthers have not yet dehisced.

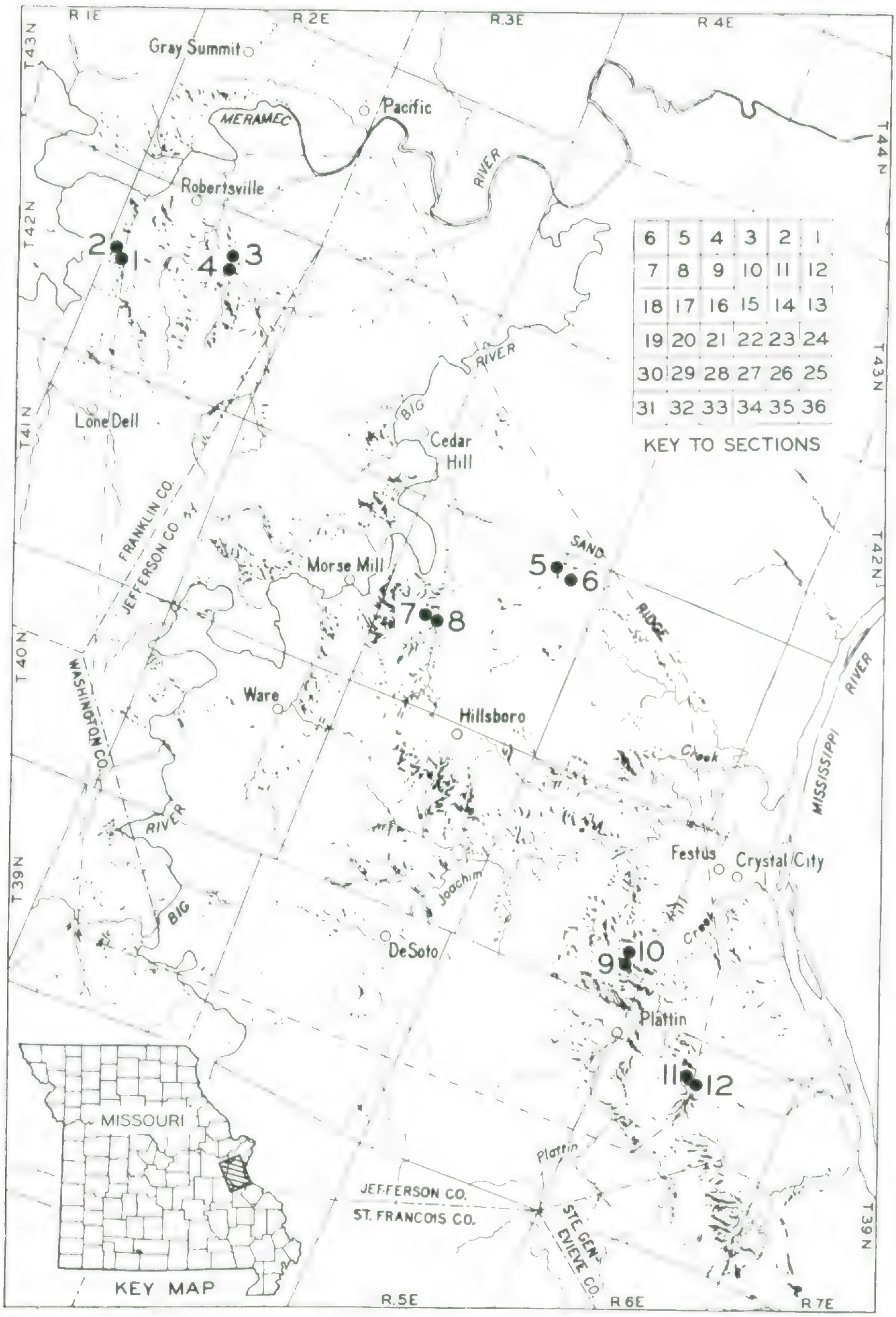

Fig. 13. Glades at which frequency of colored sepal tips has been determined. Glade numbers correspond with those of Table IV.

much pollen. No pollen grains have been detected on the pinned specimens with a hand lens. The bumblebees are undoubtedly queens who have recently come out of hibernation. In late April, they have just begun the establishment of nests (Frison, '27), and are engaged in collecting nectar rather than pollen. This is borne out by the fact that the corbiculae of all the specimens are empty. However, some pollen has been found clinging to the hairs of the head and the prothoracic legs of all of the pinned specimens. It is easy to understand how the bumblebees pick up this pollen. Their behavior at the flowers is much cruder than that of the hawk moths and the swallowtails. Instead of hanging daintily from the sepal tips and probing discretely for nectar, a bumblebee appears to be struggling in an attempt to ram its entire head into the flower as far as possible. The visits of the smaller butterflies of the Lycaenidae and Hesperiidae were puzzling at first. It was obvious that they are unable to reach the nectar by the normal route. Closer observation of several individuals showed that they insert their proboscides at the base of the flower, between the valvate margins of two sepals. By this means, of course, they completely avoid contact with the pollen. Old flowers, from which the sepals are about to drop, often swarm with black ants. The ants undoubtedly come in contact with pollen, but it is doubtful whether a single ant visits many flowers in a short period of time, or visits flowers which are young enough to have receptive styles. The honeybee specimen, *Apis mellifica,* and several of the unidentified smaller bees are well loaded down with pollen. They and the bumblebees are certainly the most important pollinators of this *Clematis*. Other species listed in Table III are probably accidental visitors; they could have been collected more efficiently by sweeping. An interesting sidelight on the insect relations of *Clematis* concerns the crab spiders (Thomisidae), of which 15 specimens were obtained. The writer was fascinated on one occasion to watch a *Hemaris* hovering before a flower, and to see it attacked and killed by a spider which had been waiting at the base of the flower.

During the 1942 season some notes were taken on the frequency of insect visits to the flowers. The observations were made without the disturbance caused by attempts to capture the visitors, and were incidental to other work. In an estimated 15 hours on seven different glades, during which an average of perhaps 20 plants were under close enough observation to insure detection of a pollinating insect, nine bumblebees were observed to visit a total of 24 flowers, two honeybees visited one flower each and flew out of sight, one small bee was observed on a single flower, five *Papilios* visited a total of 17 flowers, four *Hemaris* were observed, and one visit by an unidentified smaller butterfly was made. In all of the observations of insects, the writer has been impressed with the great variation from one glade to another. For instance, few hawk moths were recorded in 1942, while in 1943, when other glades were visited, they appeared to be the most frequent visitors, mainly because of the large numbers encountered on a single glade at R.2E, T.42N, S.15B. If any reliance can be placed on the crude estimates made above, it would seem that there is ample provision for the cross-

pollination of a plant within two days of anthesis. Actually, the frequency of pollination is probably higher; the smaller bees were not recorded because their visits failed to attract the writer's attention from other activities and no observations of nocturnal insects were made. Since the bumblebees and the honeybees are reputed to forage over wide areas, the occasional transport of pollen from one glade to another seems quite probable. While working on a single glade, the bumblebees do not systematically go from one flower to its nearest neighbor, but may fly several yards between visits. In a large colony of *Clematis* it seems probable that the circle of possible mates for a given plant may well include a few hundred individuals.

Pattern of Differentiation

Data on the distribution of gene frequencies within a population provide the most useful information for evaluating the relative roles of selection and random differentiation. However, the collection of such data presupposes a basic fund of knowledge of the genetics of an organism which exists in relatively few cases. Lacking that for *Clematis*, a careful examination of many plants has been made for a morphological character which can at least be scored as present or absent, in the hope that eventually it might turn out to have a simple genetic basis. There is considerable variation in flower color, the outer surfaces of the sepals ranging from the blue and purple of the manuals, to practically white. Most flowers in anthesis are nearly white, with considerable variation in the distribution of the small amount of color which is present. It is suspected that true albino flowers exist, but they cannot be distinguished with certainty from those in which the pigment is very dilute.

The inner (adaxial) surfaces of the recurved sepal tips, however, show a discrete variation in color which is suggestive of a simple mode of inheritance, and a number of plants have been scored for presence or absence of color (pink or blue) at this place. A collection of 36 or fewer flowers was made on each of 12 glades, so selected that they could be arranged in pairs. The two glades of a pair are on adjacent ridges (fig. 13) about 0.35 mi., or 1850 ft., apart on the average. Two pairs of collections, 4.1 mi. apart on the average, were made in each of three regions. Glades 1–4 in the Robertsville region are about 28.5 mi. from glades 9–12 in the Plattin region, and glades 5–8, in the Morse Mill region, are midway between. The number and proportion of flowers with colored sepal tips in each collection, in each pair of collections, and in each region are shown in Table IV. The proportions for the three regions, 0.36, 0.11, and 0.05, suggest a "cline" (Huxley, '38), the frequency of colored sepal tips being greatest in the Robertsville region and decreasing toward the southeast.

In examining the data statistically, the assumption that the population is really uniform in proportion of colored sepal tips has first been tested by the χ^2 test. Theoretical frequencies of colored sepal tips have been calculated by multiplying the total number of flowers in each collection by the over-all proportion,

Fig. 14. Two collections of flowers of *C. Fremontii* var. *Riehlii*, obtained at R.2E, T.42N, S.18H (A), and at R.6E, T.39N, S.4P (B), to illustrate nature of morphological variation. Scale in centimeters.

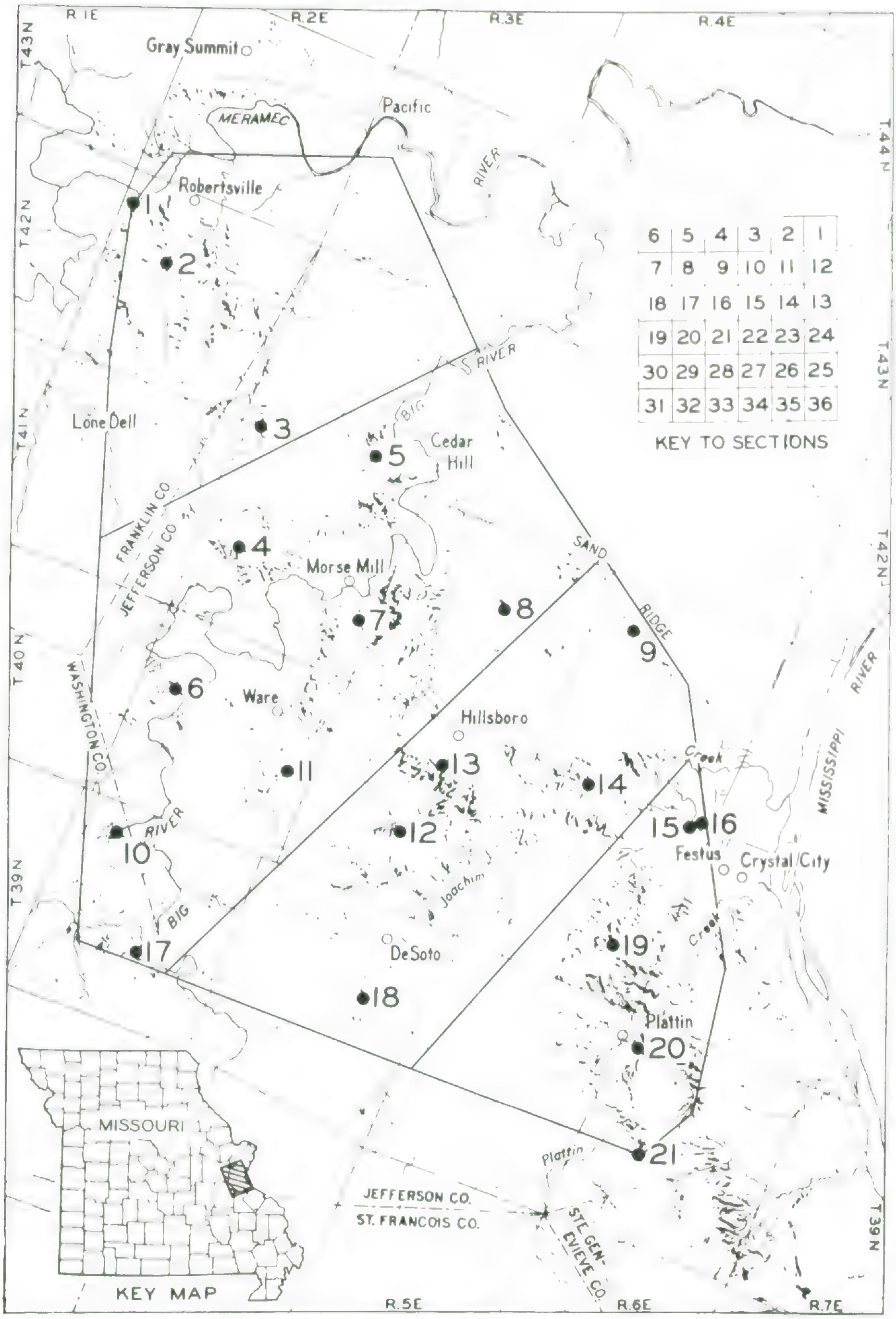

Fig. 15. Glades at which measurements have been made of sepal length, sepal width, margin width, and sepal coil. Glade numbers correspond with those of Table V.

TABLE IV

FREQUENCY OF COLORED SEPAL TIPS

(Glade numbers correspond with those of fig. 13)

No.	Location	Number of flowers			Number and proportion with colored tips			Significance of difference between proportions			
		Glade	Pair	Region	Glade	Pair	Region	Betw. glades		Betw. pairs	
								x	*2P*	*x*	*2P*
1	R. 2E, T. 42N, S. 18D	31	67	139	11–0.36	26–0.39	50–0.36	0.52	0.61	0.67	0.50
2	R. 2E, T. 42N, S. 18H	36			15–0.42						
3	R. 2E, T. 42N, S. 10E	36	72		11–0.31	24–0.33		0.52	0.62		
4	R. 2E, T. 42N, S. 10K	36			13–0.36						
5	R. 4E, T. 41N, S. 2E, F	18	54	93	3–0.17	7–0.13	10–0.11	0.57	0.58	0.81	0.42
6	R. 4E, T. 41N, S. 2G	36			4–0.11						
7	R. 4E, T. 41N, S. 20D	14	39		2–0.14	3–0.08		1.16	0.25		
8	R. 4E, T. 41N, S. 20E	25			1–0.04						
9	R. 5E, T. 40N, S. 25F	36	72	144	2–0.06	3–0.04	7–0.05	0.59	0.56	0.39	0.70
10	R. 5E, T. 40N, S. 25B	36			1–0.03						
11	R. 6E, T. 39N, S. 4P	36	72		3–0.08	4–0.06		1.03	0.30		
12	R. 6E, T. 39N, S. 4S	36			1–0.03						

Significance of difference between proportions, between regions:
Betw. glades 1–4 and 5–8: $x = 4.30$; $2P = 1.6 \times 10^{-5}$
Betw. glades 1–4 and 9–12: $x = 6.52$; $2P = 1.1 \times 10^{-10}$
Betw. glades 5–8 and 9–12: $x = 1.72$; $2P = 8.6 \times 10^{-2}$
Betw. glades 1–4 and 5–12: $x = 7.05$; $2P = 2.0 \times 10^{-12}$

0.178. Carrying through the calculation gives a χ^2 value of 43.9, with 8 degrees of freedom. The probability for a higher χ^2 value is less than 0.001, which rules out the possibility that the population is uniform in this character. (In this statistical analysis, and the succeeding ones, the methods and orthography of Rider ('39) have been followed except in the analysis of covariance on p. 446 *et seq.*)

For a more detailed analysis of the data, calculations have been made of the significance of the difference between the proportions for each pair of glades, for the two pairs of glades in each region, and for the three regions. There is no significant difference in proportion of colored sepal tips between any two adjacent glades (Table IV, third column from right), nor between the two pairs of glades in each region (Table IV, last column). The difference in proportion between the Robertsville and Morse Mill regions, between Robertsville and Plattin, and between

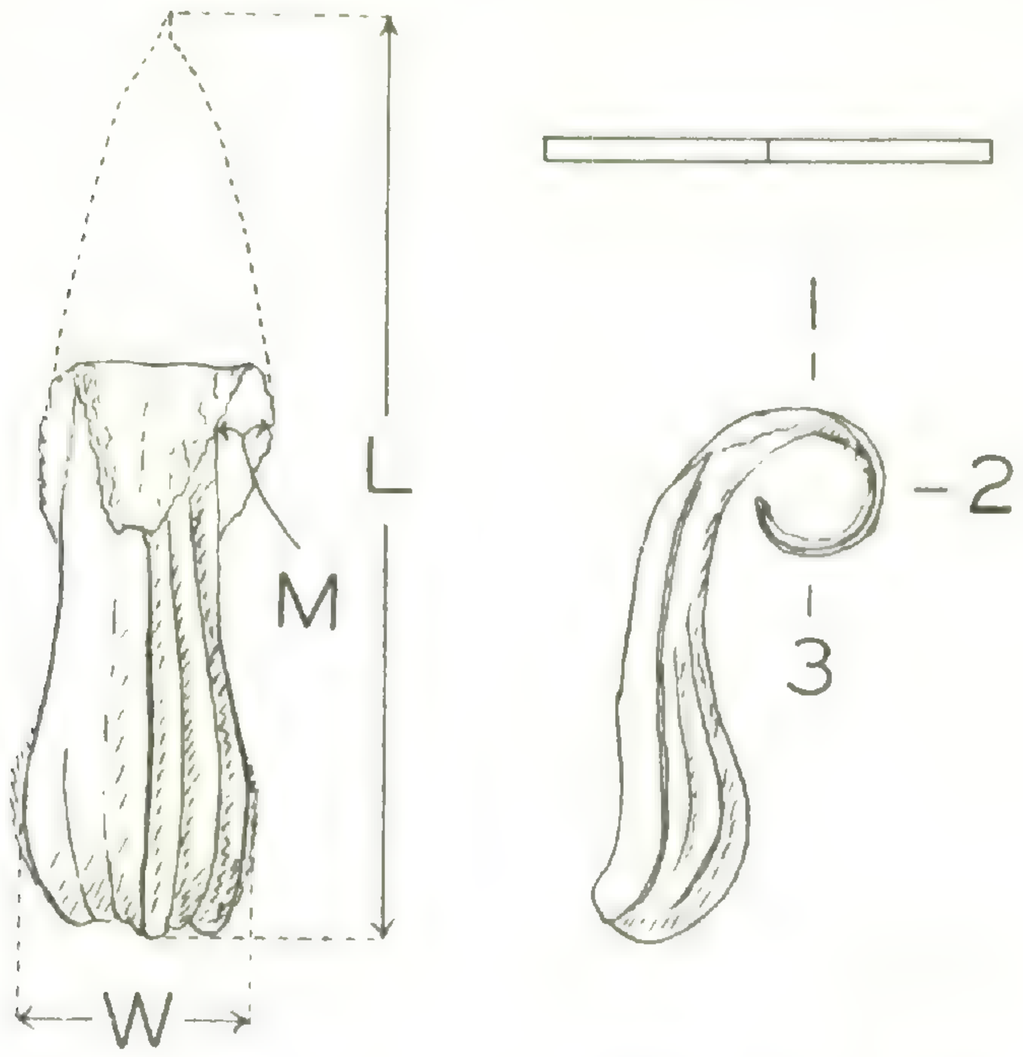

Fig. 16. Sepal of *C. Fremontii* var. *Riehlii*. Drawing at left illustrates manner in which measurements of sepal length, L, sepal width, W, and margin width, M, were made. Drawing at right illustrates method of scoring "sepal coil" in quadrants. Scale in centimeters.

the Robertsville region and the remaining glades, however, is highly significant (Table IV, bottom). Whether there is a real difference between the Morse Mill and Plattin regions is doubtful. The northwest–southeast differentiation in this character may be described as a cline, but its most significant feature is the deviation of the plants of the Robertsville region.

The flowers were brought to one place so that they could be studied at one time and photographed. Examination of the flowers indicated that the differentiation in proportion of colored sepal tips is correlated with similar differentiation in the color of the entire sepal. Perhaps it is merely an expression of the latter.

The flowers also show evident differences in a number of continuously varying characters, as illustrated in fig. 14. Measurements of four such characters have been made on another series of glades. The 21 glades at which measurements, usually of 35 flowers, were made are indicated in fig. 15. They are scattered throughout the distribution range. Measurements of the distance between each of the 210 pairs of glades have been made with dividers on a map (scale: ¼ in. = 1 mi.), averaged, and the average converted to miles. This yields an average distance between the glades of 12.8 mi.

In making the flower measurements, care was taken to select flowers only from the primary shoots of mature clumps. The flowers which terminate the primary shoots of a single clone are remarkably similar in size, coloration, and general aspect. Those which terminate secondary branches are often smaller, later in anthesis, and darker in color. None of the latter have been included in the measurements. The sampling scheme has been to select flowers from a re-

TABLE V

MEASUREMENTS OF FLOWERS

Means and Standard Deviations for Glades

(Glade numbers correspond with those of fig. 15)

No.	Location	*N*	Sepal length, mm.		Sepal width, mm.		Margin width, mm.		Sepal coil quadrants	
			$\bar{X}$	σ *	$\bar{X}$	σ *	$\bar{X}$	σ *	$\bar{X}$	σ *
1	R. 2E, T. 42N, S. 7A	45	33.56	2.93	8.84	0.54	2.03	0.47	3.29	1.05
2	R. 2E, T. 42N, S. 17B	37	34.08	3.86	9.51	1.04	2.46	0.57	3.41	1.12
3	R. 3E, T. 42N, S. 31D	35	37.54	4.92	10.00	0.98	1.87	0.70	3.06	0.88
4	R. 3E, T. 41N, S. 20A	35	34.20	3.95	9.31	0.93	1.74	0.51	3.46	0.98
5	R. 3E, T. 42N, S. 35B	35	33.09	4.10	9.31	1.07	1.70	0.43	3.31	0.80
6	R. 3E, T. 40N, S. 17A	35	34.20	3.47	10.11	0.97	1.73	0.41	2.74	0.79
7	R. 3E, T. 41N, S. 25D	35	34.66	2.92	9.83	1.19	1.89	0.42	3.69	0.55
8	R. 4E, T. 41N, S. 15B	35	33.14	3.38	10.11	1.15	2.00	0.42	3.20	0.60
9	R. 5E, T. 41N, S. 8B	32	29.72	5.92	9.06	1.08	1.69	0.47	3.34	0.84
10	R. 3E, T. 39N, S. 5D	35	32.91	4.02	9.54	1.39	1.66	0.60	2.97	0.79
11	R. 3E, T. 40N, S. 24B	35	34.46	3.72	9.34	1.03	2.06	0.64	2.63	0.87
12	R. 4E, T. 40N, S. 22C	35	35.06	3.10	10.14	1.28	1.76	0.43	3.11	0.84
13	R. 4E, T. 40N, S. 10E	35	34.37	4.06	9.80	0.97	1.70	0.41	2.80	0.69
14	R. 5E, T. 40N, S. 5D	35	33.80	3.34	9.54	1.19	1.67	0.49	2.77	0.87
15	R. 5E, T. 40N, S. 1B	35	32.37	2.74	9.37	1.28	1.89	0.41	2.89	0.95
16	R. 5E, T. 40N, S. 1C	35	32.66	3.73	9.49	1.09	1.90	0.53	2.71	0.90
17	R. 3E, T. 39N, S. 22B	35	34.60	3.59	10.26	1.21	2.14	0.55	3.00	1.00
18	R. 4E, T. 39N, S. 14A	35	36.57	3.40	10.23	1.48	2.09	0.55	3.17	0.96
19	R. 5E, T. 40N, S. 26B	35	33.89	7.02	9.69	1.96	1.74	0.53	2.71	1.17
20	R. 6E, T. 39N, S. 6J	35	35.97	4.00	9.49	4.09	2.01	0.44	3.14	0.99
21	R. 6E, T. 39N, S. 20B	35	33.34	2.89	9.31	1.12	1.64	0.43	2.97	0.83
Total		744	34.02	4.18	9.63	1.28	1.88	0.53	3.07	0.94

stricted portion of each glade, rather than to sample the entire population of the glade. Usually the measurements were begun at a point where the plants were abundant, and a roughly spiral course was followed, during which a flower from each mature plant encountered was measured. No records were kept of the location on the glade of the plants selected. A sepal was removed from each flower, and the measurements indicated in fig. 16 were made with a celluloid rule. The length of the sepal was measured to the nearest mm., after straightening the recurved tip, but no attempt was made to flatten the thick base. Width was

TABLE VI

MEASUREMENTS OF FLOWERS

A. Analysis of Variance for Glades

CHARACTER	Sum of squares of deviations	Degrees of freedom	Mean square deviation	*w*	*P*
SEPAL LENGTH					
Within glades	11,271.14	723	15.59	5.51	< 0.0001
Among glades	1,717.64	20	85.88		
Total	12,988.78	743			
SEPAL WIDTH					
Within glades	1,158.92	723	1.603	3.60	< 0.0001
Among glades	115.46	20	5.773		
Total	1,274.38	743			
MARGIN WIDTH					
Within glades	202.65	723	0.2803	5.52	< 0.0001
Among glades	30.96	20	1.5480		
Total	233.61	743			
SEPAL COIL					
Within glades	658.84	723	0.9113	3.16	< 0.0001
Among glades	57.66	20	2.8833		
Total	716.50	743			

B. Analysis of Variance for Regions

SEPAL LENGTH					
Within regions	12,906.32	740	17.44	1.58	0.20
Among regions	82.46	3	27.49		
Total	12,988.78	743			
SEPAL WIDTH					
Within regions	1,257.78	740	1.700	3.26	0.020
Among regions	16.60	3	5.533		
Total	1,274.38	743			
MARGIN WIDTH					
Within regions	224.85	740	0.3039	9.61	< 0.0001
Among regions	8.76	3	2.9200		
Total	233.61	743			
SEPAL COIL					
Within regions	705.44	740	0.9533	3.87	0.0089
Among regions	11.07	3	3.6887		
Total	716.50	743			

measured, to the nearest mm., at the widest point, quite near the base of the sepal, without any attempt to flatten it. The width of the expanded sepal margin was measured to the nearest 0.5 mm., at its widest point, usually quite near the tip of the sepal. The degree to which the tip of the sepal is recurved, "sepal coil," was scored by noting the number of quadrants through which the tip has moved in anthesis. Thus, if the sepal tip has turned through 360°, as has the one illustrated, it is scored as 4. The mean and standard deviation of each series of measurements are given in Table V. Because of the relative coarseness of the scale used for three of the measurements (width, margin and coil), Sheppard's correction has been applied in calculating the standard deviations.

Inspection of the table discloses differences in means from one glade to another in each of the characters. In order to determine whether the variation in these characters from one glade to another is greater than that on a single glade (in

TABLE VII

MEASUREMENTS OF FLOWERS

A. Means and Standard Deviations for Regions

Region	*N*	Sepal length, mm.		Sepal width, mm.		Margin width, mm.		Sepal coil, quadrants	
		$\bar{X}$	σ *	$\bar{X}$	σ *	$\bar{X}$	σ *	$\bar{X}$	σ *
Robertsville. Glades 1, 2, 3	117	34.90	4.41	9.40	1.24	2.12	0.62	3.26	1.03
Morse Mill. Glades 4, 5, 6, 7, 8, 10, 11, 17	280	33.91	3.72	9.73	1.19	1.86	0.53	3.13	0.88
Hillsboro. Glades 9, 12, 13, 14, 18	172	33.98	4.18	9.77	1.29	1.78	0.50	3.03	0.87
Plattin. Glades 15, 16, 19, 20, 21	175	33.65	4.57	9.47	1.38	1.84	0.49	2.89	0.99
Total	744	34.02	4.18	9.63	1.28	1.88	0.53	3.07	0.94

B. Tests of Significance of Differences between Means for Regions

Between Regions	Sepal length, mm.		Sepal width, mm.		Margin width, mm.		Sepal coil, quadrants	
	t	*2P*	*t*	*2P*	*t*	*2P*	*t*	*2P*
Robertsville and Morse Mill	2.28	0.023	−2.46	0.015	4.15	< 0.0001	1.12	0.26
Morse Mill and Hillsboro	−0.18	0.86	−0.33	0.74	1.64	0.10	1.06	0.29
Hillsboro and Plattin	0.70	0.48	2.08	0.038	−1.04	0.30	1.49	0.14
Robertsville and Hillsboro	1.79	0.075	−2.40	0.017	5.09	< 0.0001	1.65	0.10
Morse Mill and Plattin	0.67	0.50	2.13	0.033	0.55	0.58	2.68	0.0077
Robertsville and Plattin	2.32	0.021	−0.42	0.67	4.32	< 0.0001	2.64	0.0088

other words, whether these samples must be regarded as representing a number of separate populations or as portions of a single statistical population), the data have been subjected to an analysis of variance. The results are presented in Table VI, A. In each of the four flower characters the probability that the 21 series of measurements can be regarded as portions of the same statistical population is very low, clearly beyond the threshold of significance. This excess of variance among glades over that within individual glades is evidence of considerable local differentiation. It may be ascribed to the partial isolation of the glades, which has been discussed above.

It is also of interest to inquire whether these data demonstrate a cline, or regional differentiation of any sort, in any of the characters. In view of the local differentiation, it has not been possible to find evidence of differentiation on a regional scale by examining the means of individual glades, or by studying a series of ideograms, such as Anderson ('36) prepared from his data on *Iris*. The

sampling scheme used in selecting these glades leaves a good deal to be desired when it comes to investigating the question of regional differentiation. Nevertheless, the data for the 21 glades have been combined into four groups as shown in fig. 15 and Table VII, A. The four groups correspond approximately with the Robertsville, Morse Mill, Hillsboro and Plattin regions, described above. An analysis of variance has been carried out for each of the four sepal measurements (Table VI, B). It indicates that the excess of variance from one region to another over that within regions is significant for margin width and sepal coil, perhaps so for sepal width, and not for sepal length. In other words, there appears to be significant regional differentiation in two (or three) of the four measurements.

It is then worth while to compare the means for each of the regions. Means and standard deviations for each of the measurements have been entered in Table VII, A. The *t* value for the difference between each pair of means has also been determined. In this calculation the variance of the difference has been estimated separately for each pair of means. The probability corresponding to each *t* value has been found from a table of "Student's" distribution (Table VII, B). In sepal length, the plants from the Robertsville region are perhaps significantly higher than those of the other three regions, while there are no significant differences among the latter. The Robertsville, and perhaps the Plattin, plants have significantly narrower sepals than do those of Morse Mill and Hillsboro. The valvate margin of the sepal is wider in the Robertsville region than in the other three regions, and this difference is highly significant. As in sepal length, the differences in margin width among the Morse Mill, Hillsboro and Plattin regions are not significant. The sepals are most strongly recurved in the Robertsville region and least so around Plattin. The differences in this character between adjacent regions are on the border-line of significance, but the differentiation becomes significant from one end of the range to the other.

In summary, there is significant regional differentiation in each of the four flower measurements. In only one case, sepal coil, can the differentiation be described as a cline, in the sense of a consistent geographical trend. The most striking feature of the differentiation in these characters is the difference between the Robertsville plants and those of the other portions of the population. The same conclusion was drawn above from the analysis of the data on proportion of colored sepal tips (Table IV).

It has been shown above that there is greater differentiation in the flower measurements from one glade to another than on single glades. It may also be inquired whether there is local differentiation from one portion to another of a single glade. To answer this question it is necessary to obtain data on the location on a glade of the plants studied. Such data were not obtained for the plants whose flowers were measured. Laying out quadrats such as those used in population density studies is time-consuming, and it would not have been feasible during the flowering period of *Clematis* to have obtained both flower measurements and accurate locality data for any large number of plants.

On several glades, however, a leaf was collected from each plant plotted during the population density study. The leaves on a single plant vary in size and shape, though those at corresponding positions on different shoots of a single clone are closely similar. To obtain leaves from different plants which would be comparable, one leaf of the pair which subtends the first flower of the plant, or of the most vigorous shoot of a clone, was taken. The leaves of this pair are usually the longest, and comparatively, the widest ones on a shoot. On young sterile plants, however, the apical pair of leaves is usually small, and from such plants a leaf of the largest pair was taken, which was usually at the third or fourth node from the apex. The quadrat in which each leaf was collected was noted on the leaf with wax pencil, or on a small label attached to the leaf with Cellophane tape. Since the leaves are leathery in texture, it was not felt necessary to press them.

Two of the leaf collections have been subjected to measurement and statistical analysis. One of these was obtained over a continuous portion of the large glade at R.6E, T.39N, S.4-O (Glade no. 8 in fig. 2 and Table I). The entire area was laid out in 10-ft. quadrats (fig. 1, B), and a leaf was removed from each plant. Somewhat more than 1,000 leaves were collected. In some cases the label indicating the quadrat in which the leaf was obtained was lost, so that 983 leaves were available for study. Measurements of the dry leaves were made to the nearest mm. with a celluloid rule, some time after collection. Length was measured on the adaxial surface, from the tip to the point of attachment to the stem. Width was measured at the widest place, usually in the proximal half of the leaf. Many of the leaves are not plane, the adaxial surfaces sometimes being markedly concave, and rarely saddle-shaped. In all cases the rule was bent to follow the curvature of the leaf surface. (The author is indebted to John R. Melin, A. S., a U. S. Navy V-12 student, for the measurements, and for assistance in the calculation.)

It may first be inquired whether there are significant differences in the absolute length and width measurements from one portion of the area studied to another. This may be answered, as for the flower measurements discussed above, by carrying out an analysis of variance. For the purpose of the analysis, the area has been subdivided in three ways. The 10-ft. quadrats have first been combined by fours into a total of eighty-eight 20-ft. quadrats, which include an average of 11.17 measured leaves each. The number of leaves in each 20-ft. quadrat varies from 2 to 27 in a non-Poisson manner (see p. 417). Secondly, the 20-ft. quadrats have been combined by fours, with slight irregularities, into a total of twenty-four 40-ft. quadrats, which include an average of 40.96 leaves each, ranging from 9 to 80. The arrangement of the 40-ft. quadrats, and the numbers which have been assigned to them are shown in fig. 1, B. Finally, the entire area has been divided into three strips 80 ft. wide. They consist of the 40-ft. quadrats numbered 1–8, 9–18 and 19–24, and include 435, 435, and 113 leaves respectively. These strips will be referred to as 80-ft. quadrats.

For each of the three schemes of subdivision, calculations have been made of: the sums of squares of the deviations of the length and width of each leaf from

TABLE VIII

MEASUREMENTS OF LEAVES AT R.6E, T.39N, S.4-0

Analysis of Variance and Covariance

A

	Σx^2	Σxy	Σy^2	Degrees of freedom
Within 20-ft. quadrats	249,046.89	186,367.06	199,118.64	895
Among 20-ft. quadrats	77,572.11	71,742.80	76,685.39	87
For variance in length, $n_1 = 87$, $w = 3.20$, $P < 0.0001$				
For variance in width, $n_1 = 87$, $w = 3.96$, $P < 0.0001$				
Within 40-ft. quadrats	279,040.46	210,952.56	226,036.68	959
Among 40-ft. quadrats	47,578.54	47,157.30	49,767.36	23
For variance in length, $n_1 = 23$, $w = 7.11$, $P < 0.0001$				
For variance in width, $n_1 = 23$, $w = 9.18$, $P < 0.0001$				
Within 80-ft. quadrats	314,033.96	244,203.24	260,436.61	980
Among 80-ft. quadrats	12,585.04	13,906.62	15,367.42	2
Total	326,619.00	258,109.86	275,804.03	982
For variance in length, $n_1 = 2$, $w = 19.64$, $P < 0.0001$				
For variance in width, $n_1 = 2$, $w = 28.91$, $P < 0.0001$				

B

	Sum of squares of deviations	Degrees of freedom	Mean square deviation	w	P
Within 20-ft. quadrats:					
Average regression	139,462.41	1	139,462.41		
Regression differences	6,060.80	87	69.66	1.05	0.28
Residuals	53,595.43	807	66.41		
	(199,118.64)	(895)			
Among 20-ft. quadrats:					
Regression of means	66,351.55	1	66,351.55		
Residuals	10,333.84	86	120.16	1.81	< 0.0001
	(76,685.39)	(87)			
Total	275,804.03	982			
Within 40-ft. quadrats:					
Average regression	179,478.60	1	179,478.60		
Regression differences	2,419.41	23	105.19	1.53	0.050
Residuals	64,138.67	935	68.60		
	(226,036.68)	(959)			
Among 40-ft. quadrats:					
Regression of means	46,739.87	1	46,739.87		
Residuals	3,027.48	22	137.61	2.01	0.0034
	(49,767.35)	(23)			
Total	275,804.03	982			
Within 80-ft. quadrats:					
Average regression	189,900.55	1	189,900.55		
Regression differences	1,075.38	2	537.69	7.56	0.0005
Residuals	69,460.68	977	71.10		
	(260,436.61)	(980)			
Among 80-ft. quadrats:					
Regression of means	15,366.98	1	15,366.98		
Residuals	0.44	1	0.44	161.8	0.062
	(15,367.42)	(2)		($n_1 = 977$)	
Total	275,804.03	982			

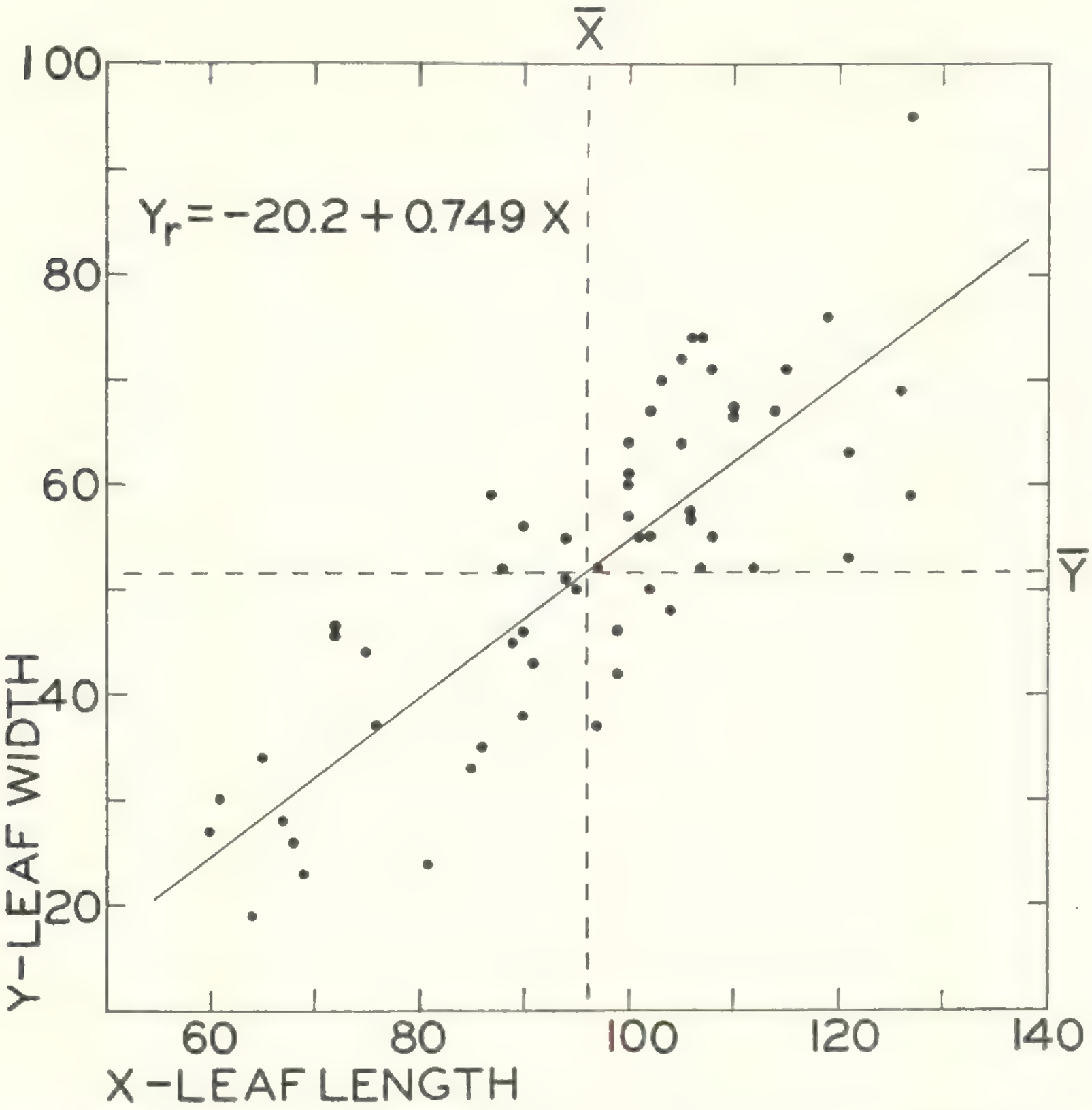

Fig. 17. Length and width measurements of 58 leaves of *C. Fremonti* var. *Riehlii* collected in 40-ft. quadrat no. 8 of fig. 1, B (R.6E, T.39N, S.4-0). Line of regression of width on length has been fitted to the data.

the mean length and width for the quadrat in which the leaf was collected (Table VIII, A, "within quadrats" rows), the sums of squares of deviations of the mean length and width for each quadrat from the over-all mean length and width ("among quadrats" rows), and the sums of squares of deviations of the length and width of each leaf from the over-all means ("total" row). Corresponding sums of products of the length and width deviations have also been determined. By the usual methods of analysis of variance, it is found that there is significantly greater variance among 20-ft. quadrats, 40-ft. quadrats, and 80-ft. quadrats, than within such quadrats, in both the length and width measurements (see the w and P values listed in Table VIII, A).

It is apparent in the field that the size of a leaf is greatly dependent on the general vigor of the plant. Presumably it is strongly influenced by environmental factors, and measurements of absolute length and width cannot be considered of much value in investigating the possibility of genetic differentiation from one portion of a glade to another. It might be supposed that the shape of a leaf is less strongly influenced by environmental variables. It is then of interest to investigate whether these data yield any information about local differentiation in

shape. One aspect of leaf shape is the relationship between width and length. One way in which this relationship might be expressed for a series of leaves is by the mean width:length ratio. When the width of each leaf is plotted against its length for a portion of the area studied, however, it appears that width and length are correlated, and that it may be justified to fit a regression line to the data (fig. 17). While the relationship between width and length may be regarded as approaching a linear one in the range of sizes at hand, the straight line fitted to the data does not pass through the origin. In other words, the width:length ratio does not tend to remain constant for leaves of varying length, but tends to increase with increasing leaf length.

Because of this circumstance, it is thought that the coefficient of linear regression of width on length is a more satisfactory index of the relationship between width and length than is the mean width:length ratio. It may be calculated by fitting an equation of the form $Y_r = a + bX$ (where Y = leaf width, X = leaf length, a = intercept on the Y-axis and b = regression coefficient) to the data for a series of leaves by the method of least squares. This has been done for the data plotted in fig. 17. (The fact that the regression line does not pass through the origin, of course, indicates that it does not fit the data entirely adequately. The relationship between width and length is undoubtedly expressed properly by a curved line passing through the origin. Nevertheless, the coefficient of linear regression is regarded as adequate for the purposes of this statistical study.)

The problem of determining whether there is local differentiation in the relationship between leaf width and length from one portion of this area to another can then be restated as the statistical problem of determining whether the regression coefficients calculated for leaves from different portions of the area are significantly different. This could be done by calculating the coefficients, and applying Student's t test to the differences between pairs. It is possible to do this more efficiently, however, by carrying out an analysis of covariance.

The total variance in width has been divided above (Table VIII, A) into two portions, that within and that among quadrats. The analysis of covariance requires that it be subdivided further. It has been suggested above that there is a significant regression of width on length within at least one of the 40-ft. quadrats (fig. 17). The variance in width within each quadrat could then be subdivided into two portions: the variance of the regression line about the quadrat mean width, and the variance of the individual width measurements about the regression line. The sum of squares of deviations in width can then be written $\Sigma (Y - \bar{Y})^2 = \Sigma (Y - Y_r)^2 + \Sigma (Y_r - \bar{Y})^2$ (where Y = width of an individual leaf, $\bar{Y}$ = mean leaf width for a quadrat, and Y_r = theoretical width for a leaf calculated by substituting its length into the regression equation for the quadrat). If the length and width measurements for a quadrat are put in terms of deviations from the quadrat mean length and width, so that $x = X - \bar{X}$ and $y = Y - \bar{Y}$, the three terms of this equation can be rewritten: $\Sigma (Y - \bar{Y})^2 =$

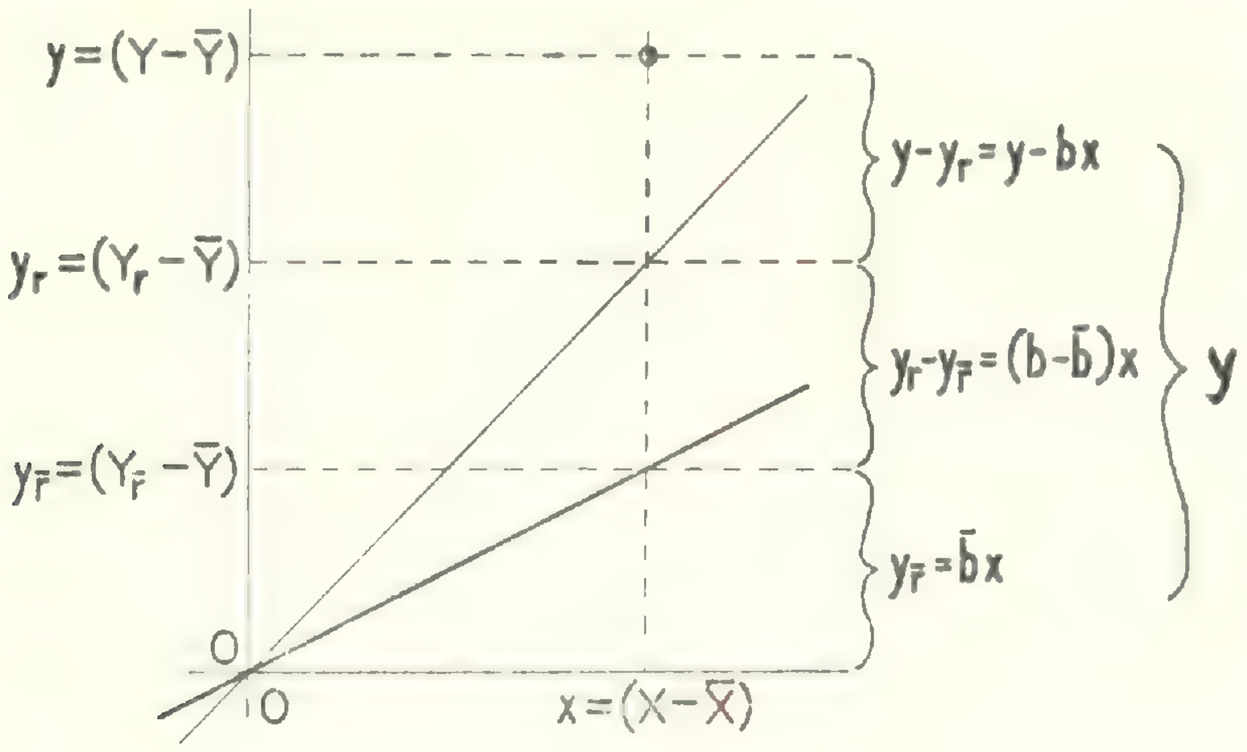

Fig. 18. Diagram to illustrate manner in which deviation in width of a leaf from its quadrat mean has been divided into three portions in the analysis of covariance. Further explanation in text.

Σy^2, $\Sigma\ (Y - Y_r)^2 = \Sigma\, y^2 - (\Sigma xy)^2/\Sigma x^2$, and $\Sigma(Y_r - Y)^2 = (\Sigma xy)^2/\Sigma x^2$, as shown in any general treatise on statistics. The equation for the regression line can also be rewritten $y_r = bx$ (where $b = \Sigma xy/\Sigma x^2 =$ regression coefficient for the quadrat).

The regression line for a quadrat, whose equation is written in this way, passes through the origin, that is, through the point which corresponds with the quadrat mean length and width. The regression lines for each of the quadrats can then be visualized as radiating from a common origin. An average regression line for a series of quadrats, which passes through the same origin, can also be considered. Its equation can be written $y_{\bar{r}} = \bar{b}x$. In fig. 18 are shown a regression line for one quadrat (lighter line) and the average regression line for a series of quadrats (heavier line). The deviation from the quadrat mean of a single measurement, y, can be seen to be made up of three portions: (1) the theoretical deviation from the quadrat mean, calculated by substituting the length deviation of the leaf into the average regression equation $(y_{\bar{r}})$; (2) the difference between the theoretical deviation calculated from the quadrat regression equation and that calculated from the average regression equation $(y_r - y_{\bar{r}})$; and (3) the difference between the actual width deviation and the theoretical deviation calculated from the quadrat regression equation $(y - y_r)$. It can be shown that the corresponding sums of squares of deviations in width for all the leaves over a series of quadrats are given by the following formulae:

(1) Sum of squares attributable to the average regression $= (\Sigma\Sigma xy)^2/\Sigma\Sigma x^2$;

(2) Sum of squares attributable to differences between the quadrat regressions and the average regression $= \Sigma\,[(\Sigma xy)^2/\Sigma x^2] - (\Sigma\Sigma xy)^2/\Sigma\Sigma x^2$; and

(3) Sum of squares attributable to deviations of the separate width measurements from the regression within each quadrat, or "within-quadrats residuals" $= \Sigma\Sigma y^2 - \Sigma\,[(\Sigma xy)^2/\Sigma x^2]$. (In each case, the first sign of summation, Σ, indicates summation over a series of quadrats, the second, summations for the series of

leaves within quadrats.)

By centering each of the quadrat regression lines at the origin, differences among the mean widths and lengths for each quadrat have been ignored. The variance in width arising from these differences has been shown to be significantly greater than that within quadrats (Table VIII, A), and it is of interest to subdivide it into two portions. The among-quadrats sum of squares of width deviations consists of:

(4) Sum of squares attributable to regression of the quadrat means, calculated most easily by substituting among-quadrats values from Table VIII, A, into an expression of the form $(\Sigma xy)^2/\Sigma x^2$; and

(5) Sum of squares attributable to deviations of quadrat mean widths from their regression, or "among-quadrats residuals," obtained by subtracting (4) from the among-quadrats sum of squares of deviations in width.

If N is the total number of leaves over the area studied, and k the number of quadrats into which the area is divided, the number of degrees of freedom to be ascribed to each of the five sums of squares is: (1) average regression, *1*; (2) regression differences, $k - 1$; (3) within-quadrats residuals, $N - 2k$; (4) regression of means, *1*; (5) among-quadrats residuals, $k - 2$; totalling to $N - 1$.

The within-quadrats residual mean square is to be regarded as the "error" mean square, with which other mean squares should be compared. The mean squares of interest in investigating leaf shape differences from one quadrat to another are: that attributable to regression differences (2), and the among-quadrats residual mean square (5). If the population were statistically uniform in the width to length relationship expressed by the regression coefficient, neither of these mean squares should be significantly greater than the within-quadrats residual mean square. (Dr. Donald R. Charles has given generously of his time in developing this scheme of analysis, in clarifying for the author the concepts involved, and in aiding in interpretation of the results of the analysis. The author, however, is responsible for this exposition of the method.)

The analysis of covariance of this leaf collection is summarized in Table VIII, B. A separate analysis has been made for each of the three schemes of subdivision of the area. It will be seen that the regression differences are not statistically significant from one 20-ft. or 40-ft. quadrat to another, but are significant among the 80-ft. quadrats. The among-quadrats residuals are significantly greater than the within-quadrats residuals from one 20-ft. or 40-ft. quadrat to another, but not among the three 80-ft. quadrats. In other words, there is statistically significant local differentiation in leaf shape from one portion of the area to another. This differentiation appears at the 20-ft. and 40-ft. levels of subdivision as significant deviations of the quadrat means from their regression, and at the 80-ft. level as differences among the within-quadrats regressions.

It may then be inquired whether this local differentiation in leaf shape, and in absolute length and width, follows any discernible pattern. For this purpose the outlines of the 40-ft. quadrats shown in fig. 1, B, have been redrawn (fig. 19). Within each quadrat outline have been placed the number of the quadrat (Q),

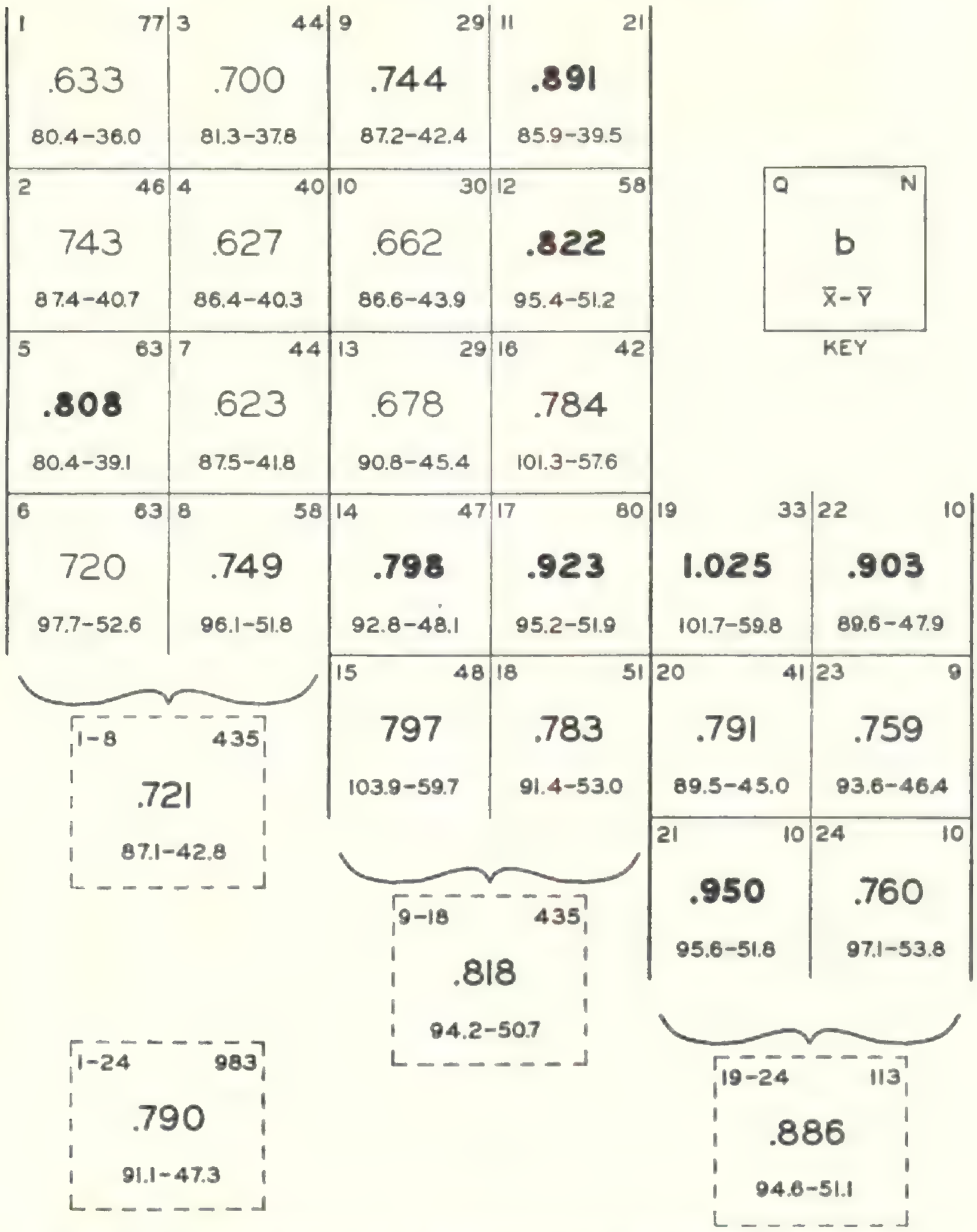

Fig. 19. Collection of leaves of *C. Fremontii* var. *Riehlii* made at R.6E, T.39N, S.4-0. Compare with fig. 1, B. Squares are 40-ft. quadrats. Number in center of each square is coefficient of regression of leaf width on length. Largest coefficients in heavy figures, intermediate coefficients in medium figures, and smallest ones in light figures. Further explanation in text.

the number of leaves measured from it (N), the regression coefficient of width on length for the leaves within the quadrat (b), and the mean length and width of the leaves ($\bar{X}$ and $\bar{Y}$). When the mean lengths and widths and the regression coefficients are compared, it is seen that the leaves of the upper-left portion of the diagram (embracing, perhaps, the nine quadrats numbered 1, 2, 3, 4, 5, 7, 9, 10 and 11, and a total of 394 leaves) are smaller and relatively narrower, than those from the remainder of the area. This grouping, however, is not without inconsistencies.

TABLE IX

MEASUREMENTS OF LEAVES AT R. 2E, T. 42N, S. 15B
Analysis of Variance and Covariance

A

	Σx^2	Σxy	Σy^2	Degrees of freedom
Within transects	90,868.35	69,857.32	76,752.97	256
Among transects	6,022.65	5,499.18	7,092.97	8
Total	96,891.00	75,356.50	83,845.94	264

For variance in length, $n_1 = 8$, $w = 2.12$, $P = 0.034$
For variance in width, $n_1 = 8$, $w = 2.96$, $P = 0.0036$

B

	Sum of squares of deviations	Degrees of freedom	Mean square deviation	w	P
Within transects:					
Average regression	53,704.56	1	53,704.56		
Regression differences	1,389.25	8	173.66	1.98	0.080
Residuals	21,659.16	247	87.69		
	(76,752.97)	(256)			
Among transects:					
Regression of means	5,021.13	1	5,021.13		
Residuals	2,071.84	7	295.98	3.38	0.0028
	(7,092.97)	(8)			
Total	83,845.94	264			

The second leaf collection which has been studied was obtained on a glade at R.2E, T.42N, S.15B (Glade no. 2 in fig. 2 and Table I). Nine transects, 10 ft. wide, were laid out at 250-ft. intervals across the glade, as illustrated in fig. 20. Collection of a leaf from each plant encountered yielded 265 leaves for measurement. The numbers for each transect range from 7 to 74. The analysis of variance and covariance described above was applied to these data also. There is significantly greater variance among transects than within in the absolute measurements of width, and perhaps of length (Table IX, A). The differences among the regressions of width on length for separate transects are not significant, but there are significant deviations of the transect means from the among-transects regression (Table IX, B). As before, this can be taken to indicate that there is statistically significant local differentiation in leaf shape from one portion of the glade to another.

In an attempt to determine whether the differentiation on this glade falls into any pattern, the transect regression coefficients have been entered near each transect in fig. 20. When the values of the coefficients are compared, it is seen that they can be arranged in two groups, those of transects 1, 3, 6, 8, and 9 being lower than those of transects 2, 4, 5 and 7. The leaves within each of these two groups of transects are statistically uniform in shape, as shown by an analysis of covariance applied to each. The glade can perhaps be thought of as divided into seven portions which have alternately wider and narrower leaves. These seven

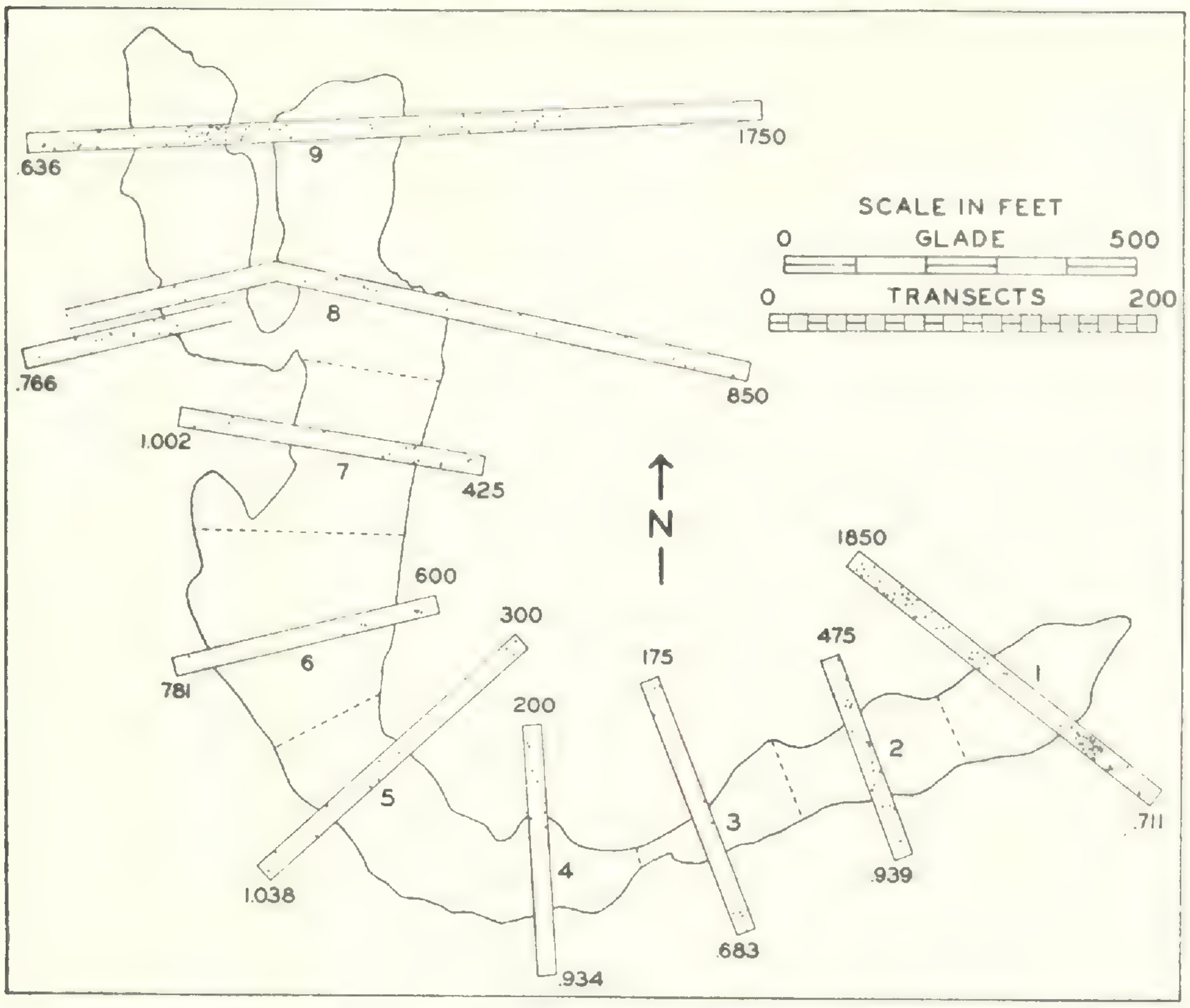

Fig. 20. Glade at R.2E, T.42N, S.15B, showing arrangement of nine transects, and distribution of plants (small dots) within transects. Length and width of a leaf from each plant have been measured. Transects have been enlarged for clarity. The fractional numbers near each transect are coefficients of regression of leaf width on length, the larger numbers have been obtained by multiplying the number of measured leaves from the transect by 25. Further explanation in text.

portions of the glade can perhaps be delimited by lines drawn midway between the transects, as has been done in fig. 20. A crude estimate of the number of plants represented by a transect is obtained by multiplying the number of leaves studied by 25, since the transects are 10 ft. wide and 250 ft. apart. These numbers have also been entered in fig. 20. If this picture of the pattern of differentiation is a true one, the differentiation appears to be effective between groups of plants numbering a few hundred.

An alternative picture of the differentiation pattern on this glade is to regard it as divided into three portions, embracing, respectively, transect 1, with an estimated 1850 plants ($b = 0.711$), transects 2–7, with 2175 plants ($b = 0.987$), and transects 8–9, with 2600 plants ($b = 0.676$). In favor of this grouping is the fact that an analysis of covariance for transects 2–7 shows no significant differentiation in shape among the five transects. If this is the true picture, the differentiation would appear to be effective between groups of plants numbering about two thousand. It does not appear possible from these data to make a choice between the two alternatives.

No information about the factors responsible for the local differentiation in

leaf size and shape can, of course, be obtained from these data. The differentiation may be merely a result of differences in environmental conditions from one portion of a glade to another, or it may have a genetic basis. The glades are not believed to be a uniform habitat for *Clematis*. This topic has been discussed on pp. 418 and 419. This non-uniformity of the glades may give rise to the local differentiation in leaf size and shape merely by inducing environmental fluctuations in a population which is essentially homogeneous genetically. On the other hand, the distribution of *Clematis* on a glade is characterized by significant aggregation (p. 419). The degree of isolation between aggregates may be such as to allow a certain amount of random genetic differentiation. It should be pointed out that, on at least one of the two glades studied, the differentiation in leaf size and shape appears to be effective over a radius including a few hundred plants. Other lines of evidence, such as the distribution pattern, and observations of pollination, have suggested an effective population size of a few hundred.

The question of an environmental, as opposed to a genetic, basis for the demonstrated local differentiation, can also be directed at the results of the analysis of flower measurements (Tables VI and VII). It appears much less probable that the differences in flower measurements from one glade or region to another are environmental fluctuations than that the leaf differences discussed above can be so accounted for. There appears to be greater variation in physical conditions from one portion to another of a single glade than between separate glades taken as wholes. Furthermore, there is undoubtedly a good deal of truth to the systematists' principle that flower and fruit characters are more "stable" than are the characters of vegetative organs such as leaves. Subjective study in the field of variation within and among clones leads one to believe that the principle holds for differences among plants as well as for differences among species.

A number of subjective observations of variation have been made, which consistently point to the existence of a considerable amount of local differentiation. The glade at R.3E, T.42N, S.31D (Glade no. 3 in fig. 15) is small and relatively isolated. It supports 75 to 100 plants. The flowers strike one immediately by their lack of color and unusual proportions. The sepals are longer (one of them measured 51 mm.), and exceed the stamens much more than usual. Perhaps one-third of the plants share these characteristics, and, in other respects as well, show a resemblance which suggests close relationship. At R.2E, T.42N, S.18H (Glade no. 2 in fig. 13) a fairly large glade with a population of 1,000 or 2,000, 15 or so plants were seen which resemble each other in that the sepals are rolled back so as to expose about half the length of the stamen mass. This peculiarity was subsequently seen on a near-by glade, but has not been noticed elsewhere. Two monstrous plants, in which the leaves are irregularly coalesced and incised, and otherwise distorted, were seen among the estimated 600 plants at R.5E, T.40N, S.13A. No teratological specimens have been seen elsewhere, though an occasional ternate shoot has been found in the midst of a decussate-leaved clone. Exploration of the large glade at R.4E, T.41N, S.2F (Glade no. 5, fig. 13)

disclosed only eight plants in a small area at one end. The six which were in flower at the time of the visit were remarkably alike in flower color and form and in general habit. The resemblance suggested that of sibs rather than of portions of a clone. Similar "family resemblances" of plants which are growing fairly close together have been seen less distinctly in many other instances.

Examination of the aerial photograph tracings and field experience both indicate that the glades are, on the whole, smaller and more isolated in the Robertsville region at the northwestern end of the range than they are farther southeast. The difference is more pronounced than the distribution map (fig. 13) suggests. An impression has grown during the field work that this difference in size and degree of isolation of separate colonies is reflected in a difference in the degree of variability in different parts of the range. The plants of the Robertsville region strike one as displaying more variation in the amount of color and pattern of color distribution in the sepals; in the width, texture, and degree of crisping of the expanded margin of the sepal; in the size, shape, and general aspect of the leaves. In general, there is a larger proportion of "queer-looking" plants than among the more uniform population of the Plattin region.

Sources of Variation

Gene mutation is generally regarded as the ultimate source of evolutionary change, and it would be desirable in studying the evolution of any organism to begin with information about the rate and direction of mutation of its genes. However, such information has been obtained for relatively few genes in a very few organisms which are favorable genetic material. Needless to say, no data whatever on this point are available for *Clematis*, and the plant is not favorable material for genetic study, because of the long period required before it reaches flowering age.

Chromosomal changes such as ploidy, inversion, and translocation have been demonstrated to be responsible for evolutionary change in several organisms. Polyploidy is practically non-existent in the genus *Clematis*. All the reported species are normal diploids ($n = 8$), with the exception of two tetraploid cultivated forms (Meurman and Therman, '39, Gregory, '41). The author has found the haploid number, $n = 8$, in several plants of *C. Fremontii* var. *Riehlii*. Examination in the field of the first division of the microsporocytes of about 75 plants has disclosed no chromatin bridges; in these plants at least, there were no conspicuous inversions.

Hybridization between species and varieties of higher plants is of rather frequent occurrence, and appears to be an important factor in the evolution of many forms. Anderson and Hubricht ('38) have studied a case of introgressive hybridization between two species of *Tradescantia*. Mangelsdorf and Reeves ('39) regard probable hybridization with *Tripsacum* as an important factor in the evolution of maize. Wide crosses are known to occur in the genus *Clematis*. *C. integrifolia*, which is fairly closely related to *C. Fremontii* var. *Riehlii*, has given

rise to *C. Durandi* by a cross with *C. Jackmani*, one of the large-flowered oriental hybrids. A hybrid of *C. integrifolia* with *C. Flammula*, one of the small-, paniculate-flowered species, is also known, and instances of hybridization between other species can be multiplied (Rehder, '40). The fact, then, that *C. Pitcheri*, which is a member of the same section (Viorna) of the genus as *C. Fremontii* var. *Riehlii*, occurs in the vicinity of the glades makes hybridization between the two species seem at least a possibility. Transfer of pollen between the two species appears possible but must be a rare occurrence. *C. Pitcheri* often occurs in the woods just below a glade, and bumblebees, at least, visit both species (Robertson, '28). However, they are separated by a difference in flowering period. *C. Fremontii* var. *Riehlii* has finished flowering by the second week of May, and *C. Pitcheri* does not come into flower until the middle of June. It continues to flower for some time, and it would probably be in anthesis when *C. Fremontii* var. *Riehlii* flowers sporadically in September.

Five plants have been found which strongly suggest that hybridization does occur. One of the plants (fig. 21, fig. 22, C) appears to be the F_1 progeny of a cross between the two forms. It grows on a glade at R.2E, T.42N, S.14H, about 3.4 mi. southeast of Robertsville. A graded farm-to-market road, surfaced with gravel, crosses the lower edge of this large glade. The supposed hybrid is rooted in the gravel embankment at the down-slope side of the road. The glade above the road is well populated with *C. Fremontii* var. *Riehlii*, a few plants persisting in the gravel at the edges of the road. A number of rather small plants of *C. Pitcheri* occur in the 300-ft. strip of woods between the road and Little Calvey Creek. The site of the hybrid is suggestive, since other species hybrids have often been reported to occur in disturbed habitats.

On the basis of morphological characters, it is impossible to regard the presumed hybrid as a member either of *C. Fremontii* var. *Riehlii* or of *C. Pitcheri*, variable as the latter species is. It appears to show pronounced hybrid vigor. Making allowances for that, it appears roughly intermediate between the two parental forms in the characters which have been examined. It has the ascending habit of *C. Pitcheri*, but the stems are considerably stouter. It appears intermediate in degree of compounding of the leaves between *C. Fremontii* var. *Riehlii*, with simple leaves, and *C. Pitcheri*, whose leaves are compound or decompound, though this character is difficult to evaluate. Its leaflets appear as thick and coriaceous as the leaves of *C. Fremontii* var. *Riehlii*, contrasting with the much thinner leaflets of *C. Pitcheri*. In *C. Fremontii* var. *Riehlii* the flowers are solitary, terminating the vegetative branches; in *C. Pitcheri* single flowers are borne on axillary peduncles, each with one pair of simple floral leaves. In the supposed hybrid both conditions occur (fig. 21). The flowers are intermediate in size between those of the putative parents. The sepals are less recurved, and their valvate margins narrower, than in *C. Fremontii* var. *Riehlii;* in these two characters the plant approaches *C. Pitcheri*. Its flowering period is a week or two later than that of *C. Fremontii* var. *Riehlii*, and earlier than that of *C. Pitcheri*. The clusters of

Fig. 21. Plant which is presumed to be the F_1 progeny of a cross between *C. Fremontii* var. *Riehlii* and *C. Pitcheri*. Note the old flower terminating the primary stem from which sepals and stamens have fallen. Scale in centimeters.

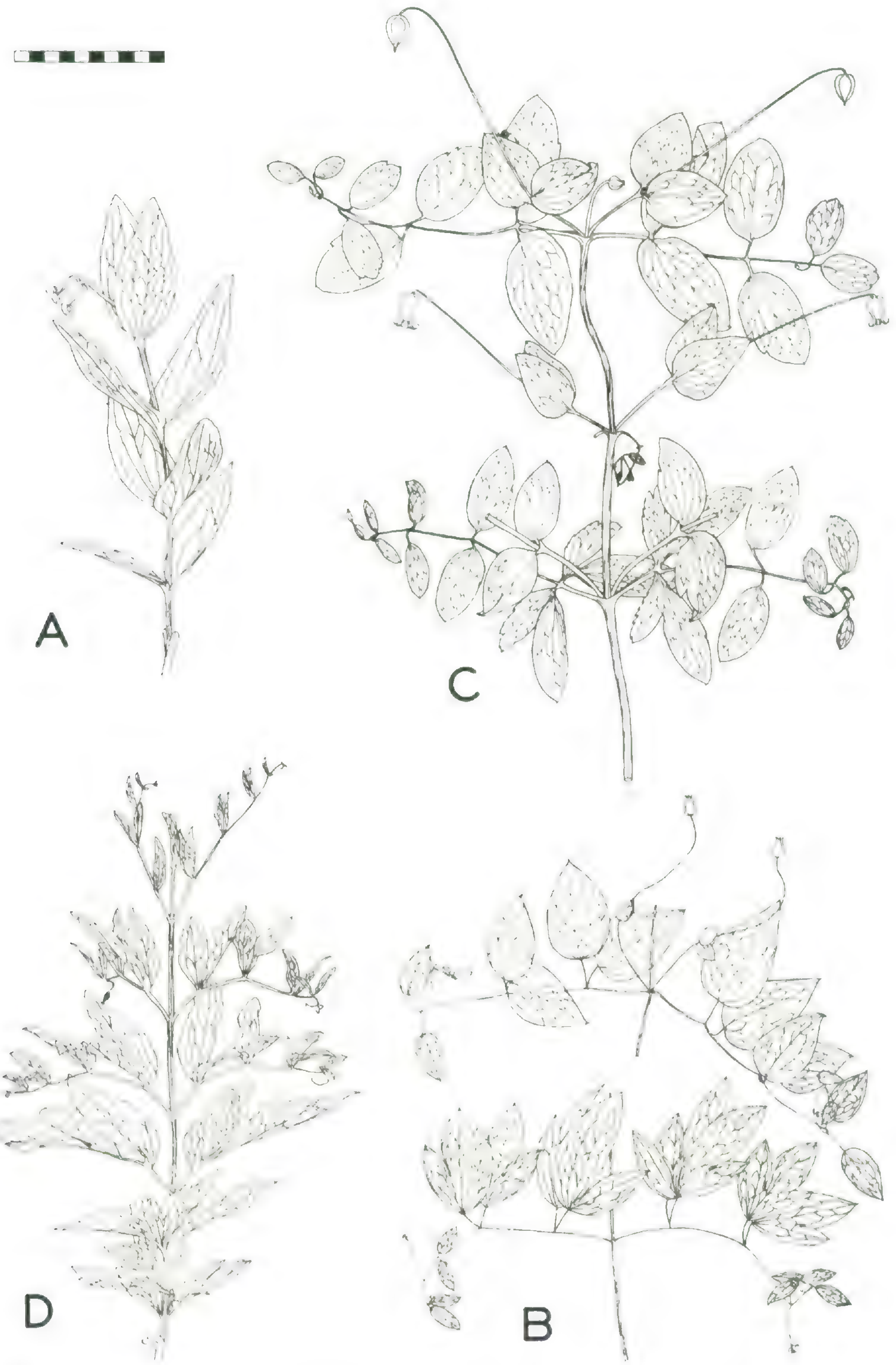

Fig. 22. *C. Fremontii* var. *Riehlii*, *C. Pitcheri* and two plants which are presumed to be the result of hybridization between them. A. Tracing of a photograph of a young plant of *C. Fremontii* var. *Riehlii*. B. Tracing of two pressed fragments of a large plant of *C. Pitcheri*. C. Tracing of a portion of a pressed plant, which is presumed to be the F_1 hybrid between *C. Fremontii* var. *Riehlii* and *C. Pitcheri*. Another shoot of the same clone has been illustrated in fig. 21. D. Tracing of a photograph of a plant which is presumed to have resulted from back-crossing of the F_1 to *C. Fremontii* var. *Riehlii*. Scale in centimeters.

achenes, and the achenes themselves, are larger than in either of the supposed parents. The achene-tails are naked as in both *C. Fremontii* var. *Riehlii* and *C. Pitcheri*.

It has not been possible to compare the plant with a *C. Fremontii* var. *Riehlii* × *C. Pitcheri* hybrid of known parentage.

The evidence at hand indicates that the supposed hybrid is fertile, though the crucial test of germinating the seeds has not been made. Several full heads of achenes have been seen, and the seeds appear viable on examination. The pollen appears normal in the microspore stage. Five microsporocytes at metaphase I have been analyzed completely. In each of them there appear to be eight normal bivalents. A larger number of cells at this stage have been examined more briefly, and all appear normal. Chromatin bridges have been seen in two microsporocytes out of about 50 at late anaphase I. These observations suggest that there may be one or more inversions differentiating the parents of the supposed hybrid, but that pairing is sufficiently normal to allow formation of good seed.

The supposition that the hybrid between *C. Fremontii* var. *Riehlii* and *C. Pitcheri* is fertile, is consistent with finding of the four other aberrant plants. They all resemble *C. Fremontii* var. *Riehlii* more closely than does the supposed F_1 plant discussed above, but are clearly outside the normal limits of variability of the former. They are not uniform among themselves, and can be arranged in a series according to the degree in which they resemble *C. Fremontii* var. *Riehlii*. Of these four plants, one found at R.2E, T.42N, S.7A and one at R.2E, T.42N, S.15B resemble the F_1 most closely. Next in order is a second plant found at R.2E, T.42N, S.7A (fig. 22, D), and the plant found at R.3E, T.41N, S.1D is nearest to *C. Fremontii* var. *Riehlii*. They suggest a series of backcrosses of the F_1 to *C. Fremontii* var. *Riehlii*.

If these suppositions are correct, some introgression of *C. Pitcheri* genes into the *C. Fremontii* var. *Riehlii* population presumably occurs. It is estimated that the number of plants which have been seen at close enough range to detect such aberrant forms as the five described above is of the order of 10,000. These figures indicate, to a first approximation, the frequency of the presumed introgression. The five plants discussed above appear to have a considerable amount of *C. Pitcheri* germ-plasm; it might also be expected that a larger number of plants would exhibit the presence of a smaller amount. It is not known, of course, in what way small amounts of *C. Pitcheri* germ-plasm might be evidenced. The most striking difference between the two species is the contrast between the simple leaves of *C. Fremontii* var. *Riehlii* and the compound leaves of *C. Pitcheri*. Occasionally a plant is seen which departs from the norm in a coarse toothing of the larger leaves, which are usually entire. It may be that this is evidence of some *C. Pitcheri* genes.

It might also be expected that introgression occurs in the converse direction. The fact that *C. Pitcheri*, *C. Fremontii*, and *C. Fremontii* var. *Riehlii* are the only plants in the genus lacking plumose achene tails is suggestive of exchange of genes between the species over a long period of time.

Another possible source of variability deserves mention. The data on frequency of colored sepal tips (Table IV), the measurements of sepal characters (Table VII), and subjective field observations lead to the conclusion that the plants of the Robertsville region diverge more greatly from the norm for the entire population than do those of the other three regions. The Robertsville glades as a group are relatively isolated, as can be seen by reference to one of the maps, (*e. g.*, fig. 2). It may be that this relative isolation is sufficient to account for the singularity of the Robertsville plants. One is inclined, however, to speculate on the possibility that the population of *C. Fremontii* var. *Riehlii*, limited as it is, may at one time have consisted of two smaller groups. One would suppose that the two groups were centered near Plattin and near Robertsville, since *Clematis* appears most abundant in these regions at present. Their merger may have taken place rather recently, in view of the presumed increase in numbers since white settlement of the Ozarks. This possibility has great evolutionary importance. If the population were at one time divided into two wholly isolated groups, considerable divergence between them would presumably have occurred. The hybridization resulting from their reunion would provide a source of variation of greater magnitude than that provided by gene mutation governed by the statistical mechanism which Wright hypothesizes, and of somewhat different nature than that provided by introgression of *C. Pitcheri* genes.

The variation which is seen in this *Clematis* population could well be the resultant of these three factors. Introgressive hybridization with *C. Pitcheri* is likely. It probably does not occur with great frequency, but genes of adaptive value in the glade habitat may occasionally be introduced into the population by this means. Isolation of the Robertsville region has allowed it to evolve to some extent along its own course, whether one considers the partial isolation of the present, or the possibly complete isolation of some past period. The supposition that some random differentiation of partially isolated groups of plants on separate glades or portions of glades occurs by the mechanism which Wright has described, is consistent with the pattern of distribution of the plant, and with the statistical pattern of variation in several morphological characters. Since it is probable that the effective population size is comparatively large, the fate of individual genes is probably not wholly a random matter, but is under some selective control.

The concept of this *Clematis* population which emerges is not that of an "old" endemic in which evolutionary change has ceased and which is doomed to extinction, though its restricted range may suggest such a picture to some minds. It is rather that of a population which has undergone marked changes in range and in numbers, and which appears to be increasing in numbers at present; one in which evolutionary changes of several sorts are occurring, though perhaps not as rapidly as in many organisms. *C. Fremontii* var. *Riehlii*, because of its presumably low competitive vigor, is probably doomed to restriction to the glade habitat. Its breeding structure is neither that of approximate panmixia which leads to extreme

specialization, nor of extreme restriction in numbers which leads to wholly non-adaptive differentiation. This being so, it may be expected to continue to thrive on the glades, and perhaps to extend its range, though the colonization of new glades will probably be slow.

Summary

Clematis Fremontii var. *Riehlii,* which is wholly restricted to dolomitic barrens, or glades, in an area of about 400 sq. mi. in east-central Missouri, has been studied in the field with particular attention to features of its distribution, biology, and pattern of variation, which are of evolutionary importance.

The population, estimated at 1,500,000, is organized into a hierarchy of natural subdivisions: *regions* of glade concentration; *clusters* of glades; *colonies* of the plant, which correspond approximately with glades; and *aggregates* of a very few, to perhaps a thousand, plants on each glade. There is great inequality in number of plants from one *colony* or *aggregate* to another. Both types of subdivision exhibit partial isolation, of a degree which is regarded as favorable for continuing evolution. The plant appears to be remarkably stable in numbers, but there is indirect evidence that it has increased since the white settlement of the Ozarks.

Inefficient seed dispersal and the longevity of the plants are factors which probably tend to promote a high degree of inbreeding. Counteracting them is the pollination of the plant by wide-ranging insects, which tends to promote cross-breeding.

Statistical study of morphological variation shows significant local differentiation at three levels of the distributional hierarchy: from one region to another, in five flower characters; from one glade to another, in four flower characters; and from one portion of a glade to another, in leaf shape. The most significant feature of the regional differentiation is the singularity of the plants near Robertsville, at the northwestern end of the range.

The pattern of distribution; the biological factors of pollination, seed dispersal and germination, and longevity; and the nature of variation in leaf shape are consistent in suggesting that the effective population size is a few hundred.

There is evidence that introgressive hybridization with *C. Pitcheri* occurs. This, together with differentiation on a regional scale, and local differentiation of a moderately random nature appear to be the most significant evolutionary processes occurring in the population.

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